A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA

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DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled **"A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA"** submitted towards partial fulfillment of the requirements for the award of **MD IN DERMATOLOGY** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr. Sukhjot Kaur,** Professor & Head, Department of Dermatology, **Dr. Sunil K Gupta**, Former Professor & Head, Department of Dermatology, & **Dr. Ekta Bansal,** Professor & Head, Department of Biochemistry. No part of this thesis has formed the basis for the award of any degree previously.

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Dedicated to

my family

my friends &

Teachers

"*The Guru is the means of realisation. There is no knowledge without a teacher."*

-Swami Vivekananda

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Dr. Stephen

LIST OF ABBREVIATIONS

AA - ALOPECIA AREATA

AAPI - ALOPECIA AREATA PROGRESSION INDEX AAPS - ALOPECIA AREATA PREDICTIVE SCORE AASI - ALOPECIA AREATA SEVERITY INDEX ADTR – ACUTE DIFFUSE AND TOTAL ALOPECIA

ALODEX - ALOPECIA DENSITY AND EXTENT SCORE APCs – ANTIGEN- PRESENTING CELLS

AT - ALOPECIA TOTALIS

AU - ALOPECIA UNIVERSALIS BMI - BODY MASS INDEX

CRH - CORTICOTROPIN RELEASING HORMONE

CRHR1 - CORTICOTROPIN RELEASING HORMONE RECEPTOR 1 CTLA4 - CYTOTOXIC T-LYMPHOCYTE ASSOCIATED PROTEIN 4 DNA -DEOXYRIBONUCLEIC ACID

DPCP – DIPHENYLCYCLOPROPENONE

ELAM-1 - ENDOTHELIAL LEUCOCYTE ADHESION MOLECULE-1

HF - HAIR FOLLICLE

HLA - HUMAN LEUKOCYTE ANTIGEN

HPA - HYPOTHALAMIC-PITUITARY-ADRENAL

ICAM-1 - INTERCELLULAR ADHESION MOLECULE-1 IDO - INDOLEAMINE 2,3 - DIOXYGENASE

IFN-γ - INTERFERON GAMMA

IKZF4 - IKAROS FAMILY ZINC FINGER 4 IL-10 INTERLEUKIN 10

IL-15 - INTERLEUKIN-15 IL2 – INTERLEUKIN 2 IL21 - INTERLEUKIN 21

IL2RA - INTERLEUKIN 2 RECEPTOR SUBUNIT ALPHA IP – IMMUNE PRIVILEGE

JAK/STAT - JANUS KINASE/SIGNAL TRANSDUCERS AND ACTIVATORS OF TRANSCRIPTIONAL SIGNALING

MHC - MAJOR HISTOCOMPATIBILITY COMPLEX

MIF - MACROPHAGE MIGRATION INHIBITORY FACTOR
MIF - MACROPHAGE MIGRATION INHIBITORY FACTOR NK – NATURAL KILLER
OCT - OPTICAL COHERENCE TOMOGRAPHY OS – OXIDATIVE STRESS
PBMCs - PERIPHERAL BLOOD MONONUCLEAR CELLS PDE4 - PHOSPHODIESTERASE-4
PRDX5 - PEROXIREDOXIN 5
ROS - REACTIVE OXYGEN SPECIES
RRMS - RELAPSE REMITTING MULTIPLE SCLEROSIS SALT - SEVERITY OF ALOPECIA TOOL
SBN - SCALP BEARD NAIL SCORE
SLE - SYSTEMIC LUPUS ERYTHEMATOSUS SNPs - SINGLE NUCLEOTIDE POLYMORPHISMS STX17 – SYNTAXIN 17
TGF-β1 - TRANSFORMING GROWTH FACTOR-BETA 1 TNF-α - TUMOR NECROSIS FACTOR ALPHA
Tregs - REGULATORY T CELLS UV – ULTRAVIOLET
VDR - VITAMIN D RECEPTOR
VIP - VASOACTIVE INTESTINAL PEPTIDE
α-MSH - ALPHA-MELANOCYTE-STIMULATING-HORMONE

ABSTRACT

Introduction: Alopecia areata (AA) is the most common form of non-scarring alopecia involving any hair bearing area. AA is an organ-specific autoimmune disease characterized by T-cell infiltrates and cytokine production around anagen stage hair follicle. Vitamin D deficiency has been hypothesized as a risk factor for the development of AA.

Aim of Study: To Study the Co-relation of Vitamin D levels in patients of Alopecia areata

Materials & methods: 100 Patients of alopecia areata of age 18 years or above attending Skin and VD OPD of Dayanand Medical College & Hospital, Ludhiana constituted the cases. 50 age and Gender-Matched controls included healthy individuals, healthy attendants of patients, patients of the skin diseases known to have no effect on vitamin D levels and their vitamin D levels were estimated. Alopecia areata was graded based on SALT's score.

Results: The study revealed a significant difference in Vitamin D levels between the case and control groups (p value< .001), with Vitamin D deficiency observed in 58% of patients in the case group compared to 28% in the control group. The mean Vitamin D levels in the case group were significantly lower, at 20.42 ± 10.92 ng/ml, compared to the control group, which had mean levels of 30.09 ± 16.12 ng/ml (p-value < .001)

Conclusion: Our study concludes that vitamin D levels were significantly lower in patients of alopecia areata than normal healthy individuals providing compelling evidence of a link between vitamin D levels and AA. This could ultimately lead to improved management strategies for AA, benefiting patients with this challenging and often distressing condition**.**

Keywords: Alopecia areata (AA), 25(OH) Vitamin D, severity of alopecia tool (SALT) score

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INTRODUCTION

Alopecia areata (AA) is the most common and prevalent form of non-scaring alopecia that involves any hair bearing area of the body. It was first described by Cornelius Census, and the term “alopecia areata” was coined by Savages in 1760.[1] AA affects both adults and children, with a lifetime risk of 2% and a prevalence of 0.1%–0.2%.[2] Males and those affected in their early childhood have been demonstrated to have a more severe course of the disease.[3] Age of onset of first patch is usually before 20 years of age in about 60% of AA patients.[4] T-cell infiltrates and cytokine production surrounding anagen stage hair follicles are characteristics of AA, suggesting an organ-specific autoimmune origin.[5]

AA is associated with human leukocyte antigen (HLA-I and II) which have been linked to several other autoimmune conditions, including vitiligo, rheumatoid arthritis, diabetes mellitus, thyroiditis, pernicious anemia, and celiac disease.[6,7] Patients with AA have higher peripheral blood levels of hair follicle-specific autoantibodies, particularly against keratin 16 and trichohyalin.[8] Thus, AA is regarded as an autoimmune illness unique to hair follicles and can be brought on by environmental causes in genetically predisposed individuals.

Nail changes in AA are reported to occur between 7% and 66% of the cases.[9–11] About 29% of adults and 50% of children with AA can have nail changes.[2,10] They are more prevalent in males and in patients with severe AA.[12] Changes to one or more nails may occur either before or after hair loss. Onychodystrophy, trachyonychia, Beau's lines, longitudinal ridging, onychorrhexis, and widespread fine pitting are among the nail abnormalities that are found in patients of AA.[12,13]

As a secosteroid hormone, vitamin D is essential for preserving bone health and calcium balance.[14] It has been suggested that vitamin D may

be a risk factor for the onset of AA and the pathophysiology of AA involves vitamin D in a significant way.[15,16] The reports of AA in individuals with hereditary vitamin D-resistant rickets have offered proof.[17,18] Further evidence is also provided by the effect of Vitamin D in cancer patients undergoing chemotherapy by protecting hair follicles from chemotherapy-induced alopecia.[19] To maintain a proper hair cycle, keratinocytes must express vitamin D receptors.[20]e 1, 25-dihydroxyvitamin D3 modulates both innate and adaptive immune responses by acting as an immune modulator that targets a variety of immune cells, including monocytes, macrophages, dendritic cells, T lymphocytes, and B lymphocytes.[21]

There have been reports that vitamin D and its analogs may be utilized to treat and prevent autoimmune diseases.[22] It is essential to reevaluate the relationship between AA and vitamin D levels considering growing evidence of an association between AA and vitamin D deficiency levels for the management of this chronic illness.

The study aims to explore the relationship between alopecia areata (AA) and vitamin D levels, driven by the emerging evidence suggesting a link between AA and vitamin D deficiency. A necessary hormone for calcium homeostasis and bone health, vitamin D has been linked to the pathophysiology of AA. Vitamin D modulates immune responses by acting on various immune cells, suggesting its potential role in preventing and treating autoimmune diseases. Given these insights, reassessing the correlation between AA and Vitamin D levels is crucial for enhancing the management of this chronic condition.

AIM & OBJECTIVE

To Study the co-relation of vitamin D levels in patients of Alopecia Areata.

REVIEW OF LITERATURE

Alopecia areata (AA) is a prevalent autoimmune skin condition that results in hair loss on the scalp and other body areas.[1,9] AA increases the chances of developing psychiatric and psychological problems as well as anxiety in patients.[23,24] Hair follicle (HF) destruction caused by an autoimmune-mediated process due to a loss of immune privilege (IP) in the HF is thought to be the cause of hair loss in AA patients.[3,5] Activated effector T cells and mast cells, cytotoxic T cells (CD8+NKG2D+ cytotoxic T cells), Janus kinase/signal transducers and activators of transcriptional signaling (JAK/STAT) pathways, regulatory T cells (Tregs), immune checkpoints, and oxidative stress (OS) are all involved in AA.[24,25] But unfortunately, pathophysiology of AA is still poorly understood and we have yet to find its complete cure.

Epidemiology: AA affects both adults and children, with a lifetime risk of 2% and a prevalence of 0.1%–02%.[2,26] First episode starts before the age of 40, peaking in the second and fourth decades of life in most of the cases.[1,27]

Clinical manifestations:

Patients with AA usually have well-defined, abrupt, patchy hair loss without any symptoms.[5,28] The patches have a smooth surface devoid of any scaling or any signs of inflammation.[9] The AA can affect any hair bearing region of the body. The scalp, beard, moustache, eyebrows, and eyelashes are the most affected areas.[24] Large regions of hair loss may result from the fusion of several distinct patches.[29]

In the initial stages of the illness, white hair generally remains unaffected. However, as the disease progresses and becomes more chronic, even white hairs may show signs of involvement.[30,31] During the active stages of AA, around the margins of patchy

hair loss exhibits distinctive characteristics known as “exclamation hairs” which are the marker of activity of the disease and may warrant systemic treatment. These are short, dystrophic, fragmented hairs that taper at the proximal end and widen at the distal end.[32,33]

Depending on the severity of the condition or the pattern of hair loss, AA can be categorized as follows.[5,24,34]

Classification according to the degree or extent of hair loss:

1. **Patchy alopecia:** There can be single or multiple patches of hair loss. This is the most common variant seen. Patches are well circumscribed, oval or round shape with smooth surface. Patches have a diameter of 2 cm and grow concentrically at a rate of 1 cm every week. Patches can present anywhere in the hair bearing areas, mostly seen in scalp, beard or eyebrows.[27]
2. **Alopecia Totalis:** Near total or total loss of scalp hair.
3. **Alopecia Universalis:** Complete or nearly complete hair loss in all hair bearing body areas**.**

Classification according to the observed pattern of hair loss[3,35]:

4. **Reticular:** A pattern resembling a network is formed when several balding patches coalesce with each other.
5. **Ophiasis:** This condition causes hair loss, primarily along the occipital and temporal bone borders, in a band-like pattern. Ophiasis pattern carries poor prognosis and is mostly seen in children.
6. **Sisaipho:** The central region of the scalp is affected by widespread hair loss, leaving the outer edges of the scalp unaffected. This pattern is the opposite of ophiasis, involving the frontal, parietal, and temporal areas. It is uncommon variant, sometimes one can get confused with male pattern baldness which is the closest differential since both are non-scarring forms of alopecia and shares the same site of involvement.
7. **Diffuse Alopecia Areata:** This has a telogen effluvium-like diffuse thinning of the hair.

8. **Acute Diffuse and Total Alopecia (ADTR):** occurs in females and manifests as an abrupt, diffuse hair loss that can quickly cover the entire scalp. This is a newer variant carrying good prognosis. Sometimes goes away on its own without any medical intervention, although chances of recurrences are always there.[36]

Unusual variants:

9. **Linear Variant:** Alopecia patch manifests as a linear pattern.[37,38]
10. **Perinevoid Variant:** This is a very uncommon variant in which the area surrounding pigmentary nevi has hair loss.[39]

IKEDA classification[40,41]:

IKEDA analyzed 1989 patients of AA and assigned them to the main four arbitrary types of alopecia.

Type 1 - Common type: 80% of patients come under this group, which is the most prevalent type. AA appears to be patchy hair loss. This group has no history of endocrine disorders, hypertension, or atopy.

Type 2 - Atopic type: Patients in this type present with an Ophiasis pattern or reticulate pattern. There is a medical history of atopic dermatitis, allergic rhinitis, asthma, and seasonal patch recurrence.

Type 3 - Prehypertensive type: A reticulate pattern of hair loss is observed in patients. Parental hypertension runs in this group's family history. Progression to AT is seen in 40% of patients of this type.

Type 4 — Combined or Endocrine autoimmune type: It is the least prevalent type that develops after 40 years of age.

Pathogenesis:

AA is an autoimmune disease that targets nails and anagen phase hair follicles.[6] The precise etiology is yet unknown. A well accepted view states

that immune cells facilitate the disruption of hair follicles' immunological privilege.[3,9,29] Many genetic and environmental factors influence pathogenesis.

Genetic factors:

Strong familial correlations exist with AA. Genetic epidemiological research indicates that first-degree relatives are more susceptible to AA. Siblings, parents, and children of AA patients had an estimated lifetime risk of 7.11%, 7.8%, and 5.7% of developing AA, respectively.[42] Several chromosomes and gene loci are linked to AA making it a polygenic disease. Genome-wide linkage analysis of families of AA revealed several susceptibility loci on chromosomes 6,10,16,18.[43] There are 138 SNPs connected to AA in 8 different genomic regions.[44] These polymorphisms were found in IL2/IL21, CTLA4, IL2RA, IKZF4, NK-activating ligands, NKG2DL3, ULBP6, STX17, and PRDX5 genes associated with T cells or the hair follicle.[43,44]

Environmental Factors:

A person's genetic vulnerability, hormones, stress, food, infections, immunizations, and a host of other factors can all have a cumulative effect on the onset, pattern, and severity of hair loss associated with AA.[7,45–47]

In addition to raising the possibility of psychological and mental problems, AA itself may make patients anxious and make them avoid social situations and gatherings. Psychological stress is the environmental element that is most frequently implicated and seen among most cases of AA. According to recent research, individuals with AA exhibited higher rates of abnormal psychosocial behaviors such as hostility, anxiety, and depression.[23,48]

The outer root sheath expresses corticotropin-releasing hormone (CRH) and CRH1 receptors (CRH-R1), and the hair bulb serves as the site where

these genes are transcriptionally transcribed.[49] The completely functional peripheral counterpart of the HPA axis, which primarily regulates the skin's stress response to UV radiation exposure as well as physical, chemical, and biological damage, is present in human hair follicles.[50]

The processes by which stress can lead to the development of AA were explored using a mouse model. They have reported early catagen phase induction and premature termination of anagen, keratinocyte apoptosis in the hair bulb upregulated, and degranulation of mast cells in mice after stressful events using this paradigm.[49,50]

Immune privilege disruption in the hair follicle:

Like the anterior chamber of the eye, the testis, the placenta, the testes, the ovaries, and the blood-brain barrier in the central nervous system, the hair follicle is an immune-privileged region.[51] These areas are shielded from potentially harmful inflammatory responses brought on by putative autoantigens. In healthy individuals, immune privilege in the hair follicle is controlled by upregulating the expression of immunosuppressive factors like TGF-β1, α-MSH, IL-10, indoleamine 2,3-dioxygenase (IDO), and VIP (vasoactive intestinal peptide) and downregulating the production of MHC class I and class II molecules.[42] Furthermore, MIF (macrophage migration inhibitory factor or NK cell inhibitor) is upregulated in normal hair follicles, and NK cells are sparsely distributed, both of which contribute to the preservation of the hair follicle's immunological privilege.[51]

The primary element thought to be responsible for the development of AA is thought to be the destruction of the hair follicle's immunological privilege.[52] Due to the loss of HF's immunological privilege in AA, there is an overexpression of MHC class I and II molecules and a decrease in immunosuppressive molecules in the afflicted area.[52] There is also upregulation of adhesion molecules such as ELAM-1 and ICAM-2 in the peribulbar and perivascular region of AA-affected hair follicular epithelium. Leucocyte migration into the dermis is mediated by adhesion

molecules, MHC class I, and class II molecules. These modifications intensify the hair follicle cells' presentation of antigens and move T-cells closer to the hair follicles.[53]

Dysregulated immune system:

Immune privilege is restricted to the anagen stage of the hair cycle in the hair development cycle.[53] In the catagen phase, immune cells infiltrate the area around hair follicles and the hair follicle undergoes involution, which is mediated by apoptosis and tissue remodeling. The immune system is continuously exposed to low concentrations of antigens produced from hair follicles because of this process.[7] Dendritic and Langerhans cells can expose lymphocytes to antigens formed by cell death, which can activate autoimmunity.[54] The improper expression of co-stimulatory molecules and antigenic peptides, which can deliver antigens to APCs and trigger an immunological response that triggers the development of AA, is thought to be caused by the presumably disordered retreat of catagen.[7]

Role of JAK/STAT pathway

Interferon (IFN)-γ and interleukin (IL)-15 are two cytokines that are critical to the pathophysiology of AA and rely on the Janus kinase and signal transducers and activators of transcription (JAK-STAT) pathway.[55,56] It is thought that JAK signaling plays a role in the mediation of AA by CD8+NKG2D+ T cells. JAK3 was highly expressed in skin biopsy specimens from AA patients. The presence of phosphorylated STAT proteins in AA HF but not in normal HF further demonstrated JAK activation.[56,57]

Three elements make up the JAK-STAT pathway: Janus kinase, or [JAK], receptors, and STAT proteins. The JAK pathway's receptors are cell

surface receptors that attach to particular hormones and cytokines. Intracellular tyrosine kinases called Janus kinases are responsible for transmitting cytokine-driven signals. The JAK-STAT pathway consists of seven STAT proteins (STAT-1, STAT-2, STAT-3, STAT-4, STAT-5a,

STAT-5b, and STAT-6) and four cytoplasmic tyrosine kinases (JAK-1, JAK-2, JAK-3, and TYK-2). Following ligand-induced signaling, Janus kinases phosphorylate their tyrosine component and initiate kinase activity, which phosphorylates STAT proteins one after the other in a sequential manner. The phosphorylation of STATs causes them to dimerize and become active. These activated STATs then go to the nucleus and initiate transcription of the specific DNA region.[58]

Role of autoimmunity:

AA has been described as an autoimmune disease fueled by T cells. This notion is circumstantially supported by associations with various other autoimmune illnesses. AA is associated with human leukocyte antigen (HLA-I and II) which have been linked to several other autoimmune conditions, including vitiligo, rheumatoid arthritis, diabetes mellitus, thyroiditis, pernicious anemia, and celiac disease.[6,59,60] Patients with AA have higher peripheral blood levels of hair follicle-specific autoantibodies, particularly against keratin 16 and trichohyalin.[8] Thus, AA is regarded as an autoimmune illness unique to hair follicles and can be brought on by environmental causes in genetically predisposed individuals.

Role of Vitamin D in alopecia areata:

There are associations of Vitamin D with various autoimmune diseases. Recently, vitamin D deficiency has been reported in AA.[61] Additionally, there have been reports of beneficial uses of topical calcipotriol in the treatment of AA.[62,63] These reports tend to establish a relationship between low levels of Vitamin D and AA.

Vitamin D sources, metabolism and functions

There are two main types of vitamin D in the human body. Over 90% is made up of vitamin D3 (cholecalciferol), which the skin produces through exposure to ultraviolet B (UVB) light from 7-dehydrocholesterol. Ergocalciferol, or vitamin D2, makes up around 10% of the total. It is obtained through diet.[64,65] Both have no biological functions and are inactive forms. 25-hydroxyvitamin D 25(OH) D, which is physiologically inert at normal concentrations, is produced by the metabolic conversion of vitamin D in the liver. 25(OH) D has been considered as one of the most reliable indicator of Vitamin D levels in humans.[64,66] 25(OH) 1α-hydroxy-D further converts 25(OH) D in the kidney to the physiologically active metabolites 1, 25(OH) 2D3 (calcitriol). The 1α-hydroxy enzyme can convert inactive 25(OH) D into active 1, 25(OH) 2D in an autocrine or paracrine way. It is also widely expressed in non-kidney cells, such as immune cells.[64,67,68]

The way by which vitamin D works is by attaching itself to the vitamin D receptor (VDR), a nuclear hormone receptor that is expressed extensively in the kidney, immune system, osteocytes, and other cell types. Vitamin D- activated VDR combines with retinoid X receptor to produce a heterodimeric complex. This complex binds to the target genes' vitamin D response regions and engages in interactions with other co-regulators to impact the expression of several genes. As a result, vitamin D has several purposes and targets different organs.[64,65,69,70]

There are two categories into which vitamin D functions: the classical function, which includes maintaining the skeleton and balancing minerals and the non-classical function type includes controlling apoptosis, differentiation, cellular proliferation, adaptive immunity, anti-oxidation, and other processes.[64,65] Many other medical issues, including cancer, diabetes, hypertension, cardiovascular disease, autoimmune illnesses, and dermatological disorders, are being investigated in relation to vitamin D.[69]

Vitamin D status in AA

Numerous investigations showed that patients with AA had far lower levels of vitamin D than the control group.[15,16,71–76] According to multiple research, patients with AA had a substantially greater prevalence of vitamin D deficiency than the control group.[75,77] However, there were two reports with contradictory findings. According to a Turkish study, individuals with AA had higher serum levels of vitamin D than healthy controls, although there was no statistically significant difference in 25 (OH) D deficits between AA patients and controls. The blood samples were only taken once in the late fall and winter months, and the authors highlighted that this may be because of the general trend in their region toward lower amounts of 25(OH) D.[78] Over a 12-year follow-up period, 133 incidences of AA were found in research comprising 55,929 women in the Nurses' Health research. A prospective analysis was conducted to assess the relationship between self-reported incident AA and estimated vitamin D status. There was no discernible correlation between the risk of incident AA and a prediction score derived from serum 25(OH) D levels.[76] Two systemic reviews and meta-analyses that were released in 2018 did show that, in comparison to the control group, patients with AA had lower vitamin D levels and a higher prevalence of vitamin D deficiency.[61,79]

Study was done by Yilmaz et al in 2012 in turkey using case control method in 42 patients which concluded that there was lower serum vitamin D levels were seen in AA patients.[80]

In 2014, Mahamid et al studied in 23 patients in Israel and found lower serum vitamin D levels in patients with alopecia areata.[72]

According to a systematic review and meta-analysis conducted by Lee et al. in 2018, AA patients are more likely to have vitamin D insufficiency and have lower serum vitamin D levels than controls.[79]

Bhat et al, in India did a cross-sectional study in 2017 and found lower serum vitamin D levels.[16]

When considered collectively, the aforementioned information indicates a strong correlation between vitamin D and AA levels, pointing to a critical function for vitamin D in the disease's etiology. But the mechanism behind this association remains to be understood.

The breakdown of the HF cycle is the cause of hair loss in AA. It is unclear how vitamin D functions in HF cycling. Studies that have been published in the literature have not shown a role for vitamin D in HF cycling, despite a study in mice showing that in calbindin-D9k knockout pups, a maternal vitamin D-deficient/low-calcium diet causes transient non-cicatricial alopecia, suggesting a role for calcium and possibly vitamin D in postnatal HF cycling.[81] However, it is now widely acknowledged that VDR plays a significant role in hair cycling.[65,70] Although, this property of VDR appears to be vitamin D-independent. In the absence of 1, 25(OH) 2D3, VDR may act as a selective suppressor or de-repressor of gene expression. Inducing the onset of anagen and maintaining the cycle transition during the initiation and regeneration of HFs are important functions of Wnt/β-catenin signaling. The decrease in Wnt/β-catenin signals, which are linked to the hair cycle, may be associated with the reduction in VDR expression in AA. The disruption of HF cycling in AA is thought to be related to the lower expression of VDR in AA.[65,70,81]

An immune-mediated response is what causes the disruption of HF cycling in AA, and there is growing evidence that vitamin D affects the pathophysiological mechanisms of autoimmunity.[22,82] Consequently, vitamin D's influence on autoimmunity in the etiology of AA may influence the HF cycle.

Possible roles of Vitamin D in the pathogenesis of alopecia areata focusing on several key elements of autoimmune mechanisms have been reviewed.

Loss of immune privilege in HF

The loss of the IP of the anagen hair bulb is considered as a key element

in AA pathogenesis.[53] Increase in the Major histocompatibility complex (MHC) I and II molecules are one of the most important causes of IP collapse.[51,53] IL-15 is thought to play a role in the collapse of IP by increasing the follicular production of MHC class I and II molecules, which is highly expressed in lesional skin from individuals with AA.[55]

Research has shown that vitamin D3 greatly reduces the amount of IFN-γ secreted by human CD4+ T cells and the amount produced by human peripheral blood mononuclear cells (PBMCs) stimulated in vitro by phytohemagglutinin.[83] Higher levels of IFN-γ have been associated with lower vitamin D levels in patients with inactive SLE, as demonstrated by Kokic et al. Their findings indicated that IFN-γ levels were 150% higher in vitamin D-deficient patients compared to those with sufficient vitamin D.[84] Mrad et al. also investigated the impact of vitamin D supplementation on the immune response in patients with relapsing-remitting multiple sclerosis (RRMS).[85] While patients with normal vitamin D continued their regular medical care, those classified as vitamin D deficient received high dosage vitamin D (10,000 IU orally daily for three months). They saw a negative association between baseline serum vitamin D and IFN-γ production, as well as a decrease in IFN-γ secretion by CD4+ T cells in the vitamin D deficient group but not in the sufficient group. These results imply that vitamin D may typically help to maintain the IP of HF by regulating the synthesis of IFN-γ. Contrarily, insufficiency of vitamin D might lead to over- secretion of IFN-γ which can play a role in the collapse of IP of the anagen hair bulb.

JAK/STAT pathway

Research has indicated that vitamin D inhibits the JAK/STAT pathway. Zhang et al. used a mouse model of type 2 diabetes mellitus to observe that the gingival epithelium's expression of the JAK1/STAT3 signaling proteins was lowered by 25VD3-enhanced protein tyrosine phosphatase non-receptor type 2 expression.[86] In a functional genomics examination of

vitamin D effects on CD4+ T cells in vivo in experimental autoimmune encephalomyelitis, Zeitelhofer et al. demonstrated that vitamin D supplementation down-regulates the genes involved in the JAK/STAT signaling pathway. Important cytokines involved in the pathophysiology of AA may have their effects blocked by vitamin D's inhibition of the JAK/STAT pathway.[82]

According to these findings, vitamin D may help regulate the hyperactive self-effector T cells during the AA pathogenesis by strengthening Treg cell inhibitory function.

Investigations in AA

In doubtful cases, a scalp biopsy, fungal culture, syphilis serology, and potassium hydroxide smear may be helpful. Alopecia tool score (SALT score), dermoscopy, hair pull test, and hair pluck test are helpful in determining the disease's activity and severity.[87] A non-invasive method that has recently been used to identify the anomalies in the hair shafts of AA patients is optical coherence tomography (OCT). Bartles et al. showed that there is considerably reduced cross section of hair from an AA patch as compared to hairs from an unaffected area. Therefore, OCT could be a helpful non-invasive method to distinguish AA from other possible causes of patchy alopecia, like trichotillomania.[88] A number of indicators point to an active disease: exclamatory mark hairs at the periphery, positive hair pull test results (>6 hairs), daily hair count results (>100 hairs), hair pluck test results (more telogen hairs), and dermoscopy results (black spots, broken hair, and tapering hair). On dermoscopy, “yellow dots and short vellus” hairs are commonly observed in AA but there findings are not specific for AA as these can be seen in other hair abnormalities.[33,87,89]

Abnormalities associated with Alopecia Areata:

With Alopecia Areata, nails are most frequently affected after hair follicles; from 7% to 66% of patients are affected.[10] The most frequent nail

abnormality is nail pitting, which is characterized by fine, shallow, and grid-like pits. Trachyonychia is the second most prevalent type of nail abnormality.[13] Additional nail findings include red lunula, white nails, punctate or transverse onychomadesis, beau's lines, onychorrhexis, koilonychia, and nail thinning or thickening.[9] Nail abnormalities are more commonly seen in patients with severe Alopecia patterns and children.[4] Nail changes are the poor prognostic marker in alopecia areata and signifies active disease progression and warrants more aggressive treatment therapy.[5]

Alopecia Areata is more frequently associated with autoimmune illnesses. The most frequent autoimmune condition, autoimmune thyroid disease (thyroiditis), affects 8% to 28% of people with alopecia areata and second most common disorder is vitiligo which occurs in 3% to 8% of patients.[90,91] Ulcerative colitis, Psoriasis, lupus erythematous, multiple sclerosis, celiac disease, pernicious anemia and Addison's disease are other autoimmune diseases linked to alopecia areata.[59] Patients with AA have a two-fold increased risk of developing atopy in the form of atopic dermatitis, allergic rhinitis, and asthma in descending order.[92]

Assessment of Alopecia Areata:

Severity of AA can be measured by Severity of Alopecia Tool (SALT) score, developed by the National Alopecia Areata Foundation working committee.[93,94]

SALT Score

SALT score is useful to find out the quantitative assessment of scalp hair loss. As we can see in figure 1, Based on surface area, the entire scalp is split into four sections: the right side (18% - 0.18), left side (18% - 0.18), posterior (24% - 0.24), and top (40% - 0.4). The SALT score is obtained by adding the results of each area's products. The percentage of hair loss in each location is calculated separately and multiplied by the amount of scalp covered in that area of the scalp. If, for instance, the top, back, right,

and left sides all experience 40%, 30%, 20%, and 10% hair loss, respectively, the SALT score can be obtained as (40 × 0.4) + (30 × 0.24) + (20 × 0.18) + (10 × 0.18) = 16 + 7.2 + 3.6 + 1.8 = 28.6.

SALT score is simple to validate and reproduce in the OPD.

Limitations of SALT score[94]:

- The degree of hair loss in other areas, such as the beard, eyebrows, and eyelashes, cannot be assessed using this score.
- Nail involvement, body hair, and hair pigmentation are not included
- It fails to recognize the disease's activity.
- Observer bias

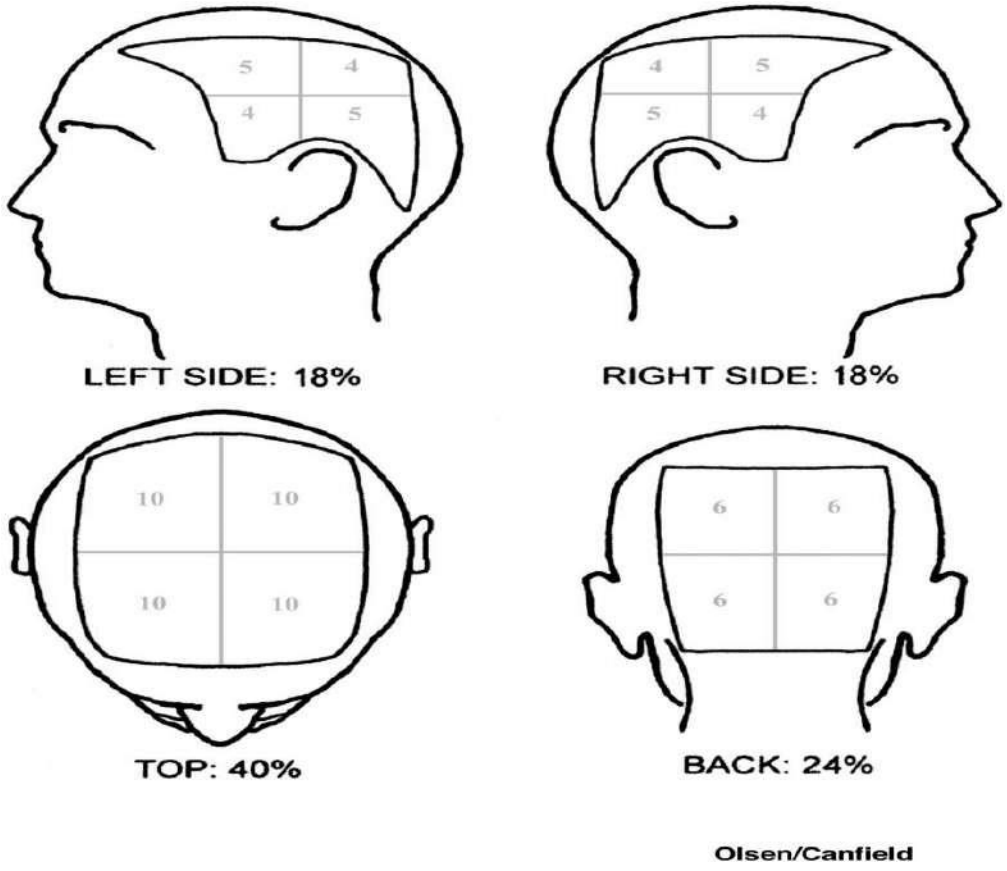


Image 1: Pictographical representation of SALT scoring

*Image 1 has been taken from study by Olsen at al [93]

Other scoring systems in Alopecia areata:

AAPI (Alopecia Areata Progression Index) score[95,96]*:*

The entire scalp region's overall level of hair loss activity is represented by this score. It is not limited to alopecic areas. In this score like the SALT score, the scalp is divided into vertex, right side of the scalp, left side of the scalp, and posterior aspect of the scalp, and each area is assigned with a multiplication factor of 0. 4,0.18,0.18 and 0.24 respectively. Dermoscopic features and the hair pull test are used to generate this score as well as the proportion of hair loss activity. The score in each quadrant is calculated by multiplying the percentage of hair loss in the given quadrant, the activity of hair loss, and the multiplication factor in the same quadrant. The total score is calculated by adding individual scores from each of the four quadrants.

AASI (Alopecia Areata Severity Index) score:

This score, known as the AASI score of the scalp, is designed to evaluate the degree of alopecia areata in the beard area, upper face, and scalp. The AASI score of the beard and the upper face are calculated independently, and the sum of the AASI scores for the three regions yields the final result.

AASI of the scalp is calculated with the help of SALT score. The maximum score given is 100.

AASI of beard region is determined by splitting the beard area into five parts: the moustache area, the regions on the right and left cheek, the regions on the right and left neck, and the region. These regions are then multiplied by 0.10, 0.20, 0.20, 0.25, and 0.25, respectively. The percentage of hair loss in each region is multiplied by a multiplication factor in the same region to attain the score of the region, and the final

score is calculated by summing up the scores in the five regions.
The maximum possible score is 100.

AASI of the upper face is determined by splitting the face into two sections: the lashes and the eyebrows. Each eyebrow has a multiplication factor of 0.15, and each eyelash has a multiplication factor of 0.10. “The percentage of hair loss in each region is multiplied by a multiplication factor in the same region to attain the score of the region, and the final score is calculated by summing up the scores in the three regions”. The maximum score is 50.

Total AASI = AASI of scalp+ AASI of beard+ AASI of the upper face.

The maximum AASI score in case of males is 250 whereas in females it is 150(not including beard hair loss).

ALODEX: Alopecia Density and Extent Score[93]

AAPS: Alopecia Areata Predictive score[97]

There is no well-defined categorization scheme for determining the severity of alopecia areata. Every classification system has its own limitations.

Course and prognosis

Although the course is unpredictable, the chance of not recovering from the initial attack is estimated to be only one is 150.[98] Spontaneous remissions are seen in about 80% of patients who present with limited patchy hair loss of less than 1 year duration.[5] Regrowth of a few terminal hairs in a patch indicates a prognosis much worse than a regrowth of fine Vell us hair.[24]

Poor prognostic indicators of AA include the following:[3,5,7]

1. Early disease onset
2. Nail changes: pits, onycholysis, onychodystrophy, anonychia
3. More than 50% of scalp involvement
4. Loss of eyebrows and eyelashes
5. Alopecia totalis
6. Aloepcia universalis
7. Regrowth of a few terminal hairs in a patchy manner within the patch
8. Other associated systemic disorders such as connective tissue disorders, hypertension, atopy.
9. Associated genetic disorders: Down syndrome[60]
10. Family history of AA
11. Recurrent episode
12. MIF-173*C gene
13. Patterns: ophiasis, sisaipho, reticular

Management of the Alopecia Areata:

General principles:

The course is uncertain. The patient's age and the degree of hair loss are the two most crucial variables to consider while choosing a treatment plan.[25] As there may be spontaneous resolution of patches in patients with limited disease who have a duration of less than 1 year but if duration is more than 1 year it is quite difficult to treat and carries a poor prognosis.[28,99] For this particular patient population, reassurance might be adequate but it should be stated that regrowth may take more than three months following development of individual patch.[24] For longstanding, refractory and extensive alopecia both systemic and localized treatment should be initiated. Treatment goals in AA include cessation of progression of the disease and to promote the hair regrowth over affected areas.[28,42]

Topical therapies:

Treatment options include topical immunotherapy using diphenylcyclopropenone (DPCP), topical minoxidil, topical bexarotene, topical tretinoin, topical anthralin, topical corticosteroids, intralesional corticosteroids, topical cyclosporine, topical capsaicin, fractional photo-thermolysis laser, excimer laser, and phototherapy which can be used topically over the affected areas.[99–101]

Systemic Therapies:

To treat AA, T-cell-driven immunological damage must be stopped to promote hair growth, and the anagen phase must subsequently be further repaired. There are several systemic therapies which are being used in alopecia areata, they are systemic corticosteroids which can be given daily or as weekly mini pulse, methotrexate, cyclosporine, azathioprine, biologic drugs such as TNF-α inhibitors, abatacept, dupilumab, secukinumab, JAK-STAT inhibitors and PDE4 inhibitors like apremilast.[57,101–105]

MATERIALS AND METHODS

Study design: Case-Control, Observational study

Study duration: One year

This is a case-control study of clinically diagnosed patients of Alopecia Areata attending outpatient department of Department of Dermatology of Dayanand Medical College and Hospital, Ludhiana. Ethical committee clearance was observed and informed consent was obtained in the regional as well as English language. The study was conducted with 100 consenting, consecutive patients aged 18 years or above, diagnosed with Alopecia Areata, and 50 age- and gender-matched healthy controls.

INCLUSION CRITERIA:

1. Clinically diagnosed patients of age18 years or above with Alopecia areata
2. Patients willing to participate in the study.

EXCLUSION CRITERIA:

1. Pregnancy or lactation
2. Woman wearing veil
3. Vitamin D supplementation
4. Alcohol consumption on a regular basis, digestive issues, bone or renal illness, sarcoidosis, or any other metabolic systemic diseases treated within the last three months with systemic steroids, barbiturates, bisphosphonates, sulfasalazine, and psoralen with ultraviolet A/UVB therapy
5. Obesity defined by body mass index (BMI) ≥25
6. Patient denying being a part of the study
7. Patient Concurrent or family history of autoimmune diseases — vitiligo, rheumatoid arthritis, diabetes, thyroid disorder, or lupus erythematosus.

Study participants:

The study involved 100 consecutive consenting patients clinically diagnosed with Alopecia Areata, aged 18 years or above, who were attending the OPD at Dayanand Medical College & Hospital, Ludhiana. These patients were selected based on the inclusion criteria and were enrolled consecutively until the target sample size was reached. Additionally, 50 age- and gender-matched healthy controls were recruited from the general population or attendants of patients, ensuring that the controls had no history of diseases that could affect Vitamin D levels or hair growth.

Methods:

Data collection involved obtaining comprehensive demographic and clinical information from all participants. For each patient, demographic details including age, gender, and duration of illness were recorded. The presence of associated conditions such as atopy and family history of similar conditions were documented. The clinical examination was done, and details of site and pattern of hair loss were recorded. The examination also included the evaluation of nail changes.

To assess the severity of Alopecia Areata, the Severity of Alopecia Tool (SALT) score was used. The SALT score quantifies the extent of scalp involvement by dividing the scalp into four regions: the right side (18% of the total scalp area), the left side (18%), the posterior (24%), and the top (40%). The percentage of hair loss in each region was calculated, and each value was then multiplied by the corresponding area percentage. The sum of these values resulted in the total SALT score, which represented the overall extent of hair loss on the scalp.

For the measurement of 25-hydroxyvitamin D levels to the clinical examination, blood samples were taken from all participants. The blood samples were analyzed using Chemiluminescence Immunoassay (CLIA) on the Beckman Access 2 system, a standard and accurate method for determining Vitamin D levels. Based on the results, Vitamin D levels were

categorized as follows: Deficient (<20 ng/mL), Insufficient (21-30 ng/mL), and Sufficient (31-100 ng/mL).

Control Group:

The control group consisted of 50 age- and gender-matched healthy individuals. These individuals were selected from the general population or as attendants of patients attending the OPD, provided they had no history of conditions known to affect Vitamin D metabolism or hair growth. Like the patient group, the control group underwent the same process of blood collection and Vitamin D level measurement.

STATISTICAL ANALYSIS:

Data has been described in terms of range; mean and standard deviation (±SD%) frequencies (no. of cases) and relative frequencies (percentage) as appropriate. comparison of quantitative variables between the study groups was done using student t test and Mann Whitney U test for independent samples for parametric and non-parametric data respectively. Chi square test was performed and exact test was used when the expected value is less than 5.A probability value (P value) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS26 (Statistical package for social science) for Microsoft windows.

OBSERVATION AND RESULTS

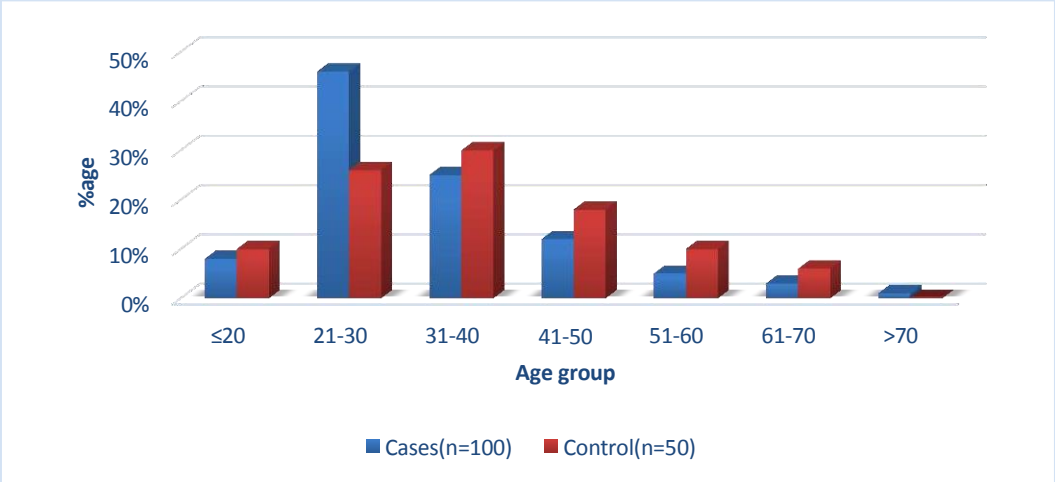
1. Age

The age distribution of the patients is depicted in Table 1. The age distribution was comparable in both cases and controls with most patients between the age of 21-30 years (cases: 46, 46%; controls: 13,26%) followed by 31-40 years (cases: 25,25%; controls: 15,30%), 31-40 years (cases: 12,12%; controls: 9.18%) (p value= .302) (Table 1;Figure 1).

Table 1: Age distribution of patients

Age groups	Cases(n=100)		Control(n=50)		Chi-square	p-value
	n	%	n	%		
≤20	8	8%	5	10%	7.213	.302
21-30	46	46%	13	26%		
31-40	25	25%	15	30%		
41-50	12	12%	9	18%		
51-60	5	5%	5	10%		
61-70	3	3%	3	6%		
>70	1	1%	0	0		
Total	100	100	50	100		

Figure 1: Bar Graph depicting the age distribution of the patients



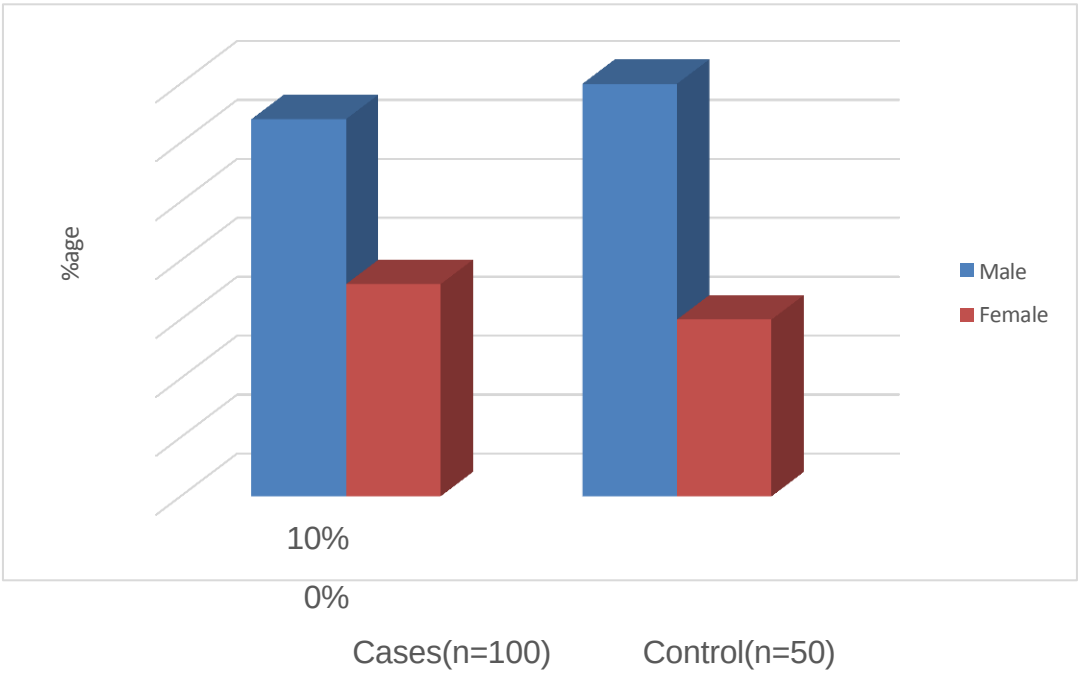
2. Gender

There were 64 (64%) males and 36 (36%) females among the cases and 35 (35%) males and 15 (30%) females among the controls. The distribution of the genders was similar, with males outnumbering females in both the groups (p value=.465) (Table 2; Figure 2).

Table 2: Gender distribution of the patients

Gender	Cases(n=100)		Control(n=50)		Chi-square	p-value
	n	%	N	%		
Male	64	64%	35	70%	.535	.465
Female	36	36%	15	30%		
Total	100	100%	50	100%		

Figure 2: Bar Graph depicting the gender distribution of the patients



3. VITAMIN D LEVELS

Comparison between cases and controls

The study revealed a significant difference in Vitamin D levels between the case and control groups (p-value <.001), with Vitamin D deficiency observed in 58% of patients in the case group compared to 28% in the control group. Vitamin D insufficiency was found in 21% of patients in the case group and 21.30% in the control group. Vitamin D sufficiency was achieved in 21% of patients in the case group, whereas 30.70% of the control group exhibited sufficient levels of Vitamin D (Table 3a, figure 3b). The mean Vitamin D levels in the case group were significantly lower, at 20.42 ± 10.92 ng/ml, compared to the control group, which had mean levels of 30.09 ± 16.12 ng/ml (p-value < .001) (Table 3b, figure 3b).

Table 3a: Distribution of vitamin D levels among cases and controls

Vitamin D deficiency	Cases(n=100)		Control(n=50)		Chi-square	p-value
	n	%	N	%		
Deficiency	58	58.00%	14	28.00%	15.407	<.001
Insufficiency	21	21.00%	11	22.00%		
Sufficiency	21	21.00%	25	50.00%		
Total	100	100.00%	50	100.00%		

Figure 3a: Bar Graph depicting the distribution of vitamin D levels among cases and controls

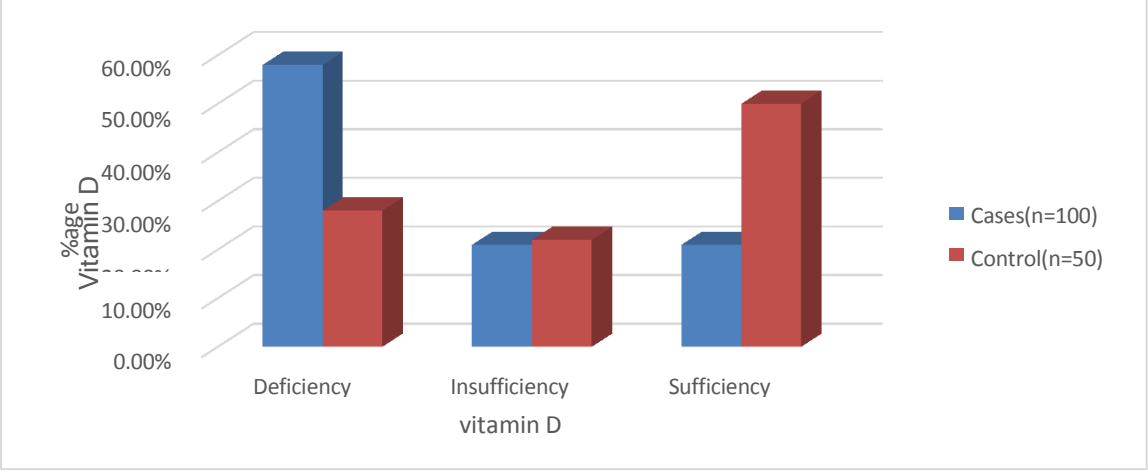
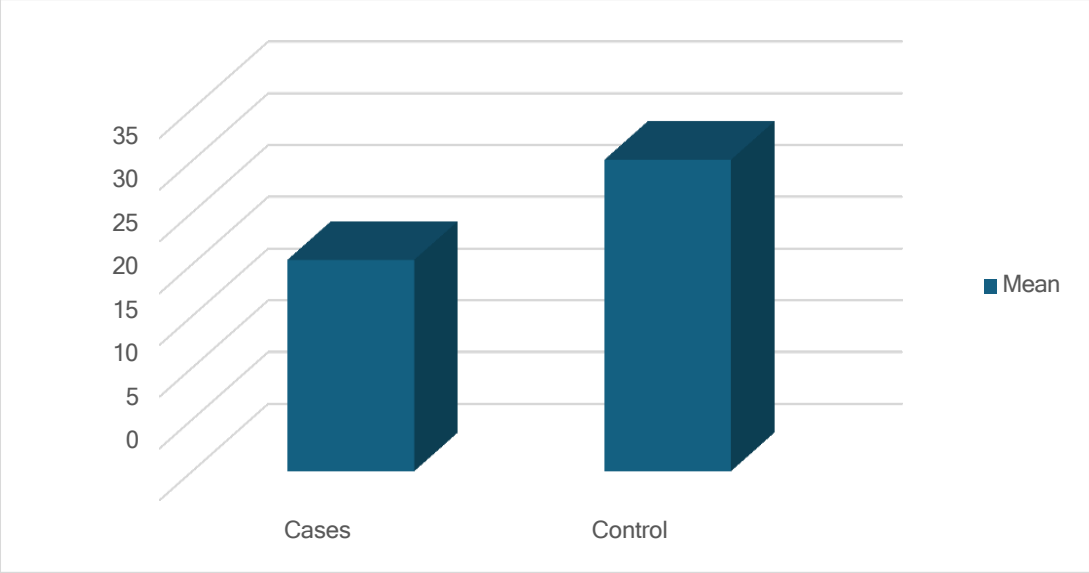


Table 3b: Vitamin D levels among cases and controls.

Vitamin D levels	Groups	No. (n)	Mean Vitamin D (ng/ml)	Std. Deviation	p-value
	cases	100	20.42	10.92	<0.001
	control	50	30.09	16.12	

Figure 3b: Vitamin D levels among cases and controls.



ALOPECIA AREATA (cases; n=100)

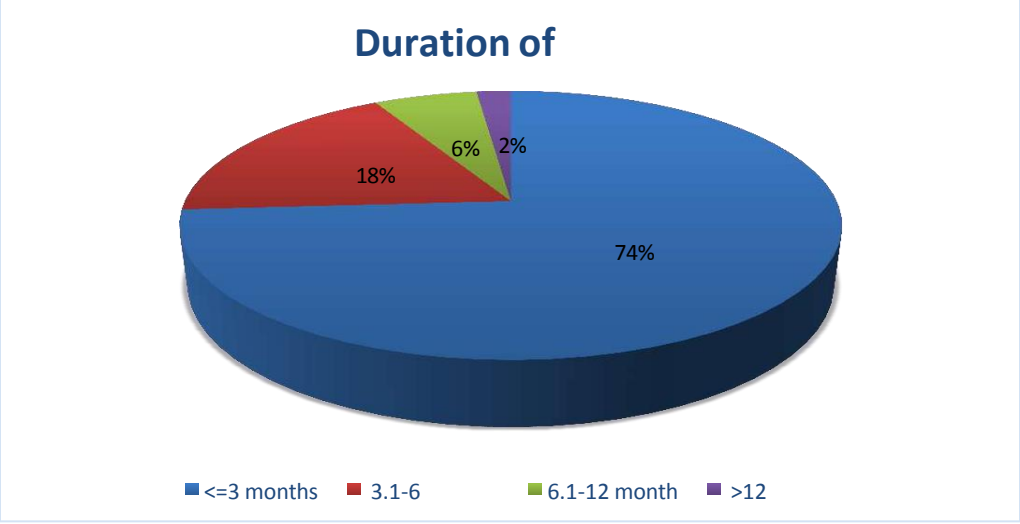
4. Duration of illness

The duration of the disease was variable in all the patients. 74 (74%) patients had a history of alopecia areata for three months or less, 18% had a history of alopecia areata lasting 3.1 to 6 months, 6% had it for 6.1 to 12 months, and only 2% had alopecia areata for more than 12 months. The mean duration of alopecia areata among patients was 3.98 ± 6.98 months(Table 4; Figure 4).

Table 4: Duration of disease (months)(n=100)

Duration of illness	Number (n)	Percent
<=3 months	74	74
3.1-6 months	18	18
6.1-12 month	6	6
>12 months	2	2
Total	100	100

Figure 4: Pie chart depicting the duration of disease (months)(n=100)



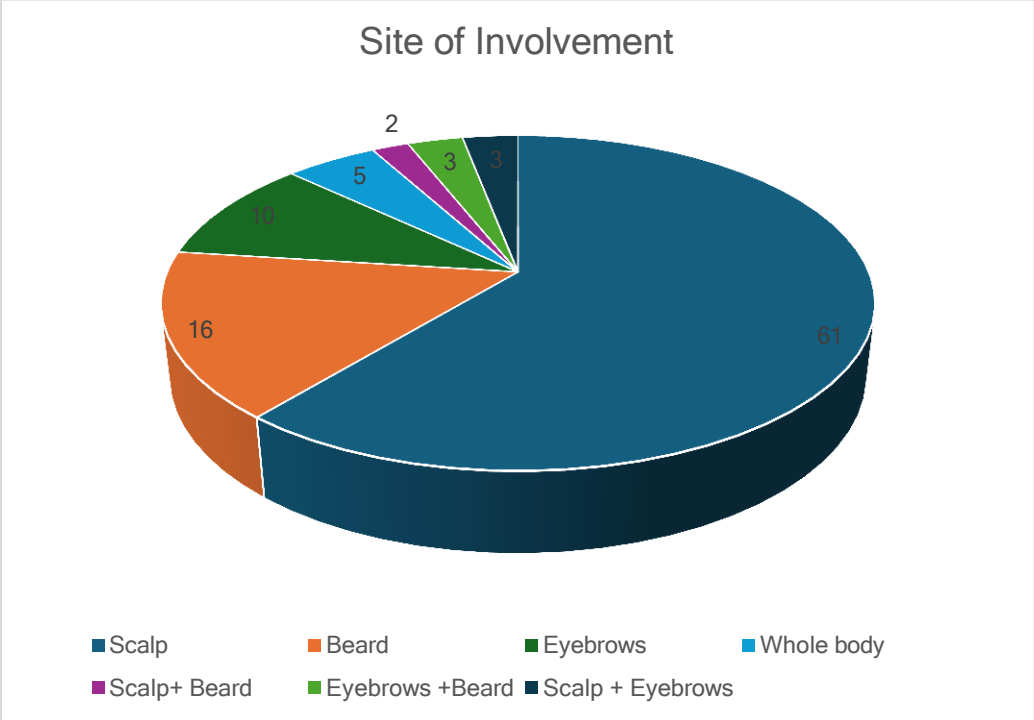
5. Site of involvement:

The distribution of alopecia areata patches among patients varied across different body sites. Most cases (61%) exhibited patches exclusively on the scalp, followed by 16% with patches solely on the beard and 10% with patches confined to the eyebrows. A smaller proportion of patients (5%) experienced patches across their entire body. Additionally, 2% of patients had patches present on both the scalp and beard, while 3% had patches on both the eyebrows and beard, and another 3% had patches on both the eyebrows and scalp. This diverse distribution highlights the variability in the presentation of alopecia areata among individuals, with certain sites being more commonly affected than others (Table 5; figure 5).

Table 5: Sites of involvement of alopecia areata (n=100)

Site of involvement	Frequency	Percent
Scalp	61.00	61.00
Beard	16.00	16.00
Eyebrows	10.00	10.00
Whole body	5.00	5.00
Scalp+ beard	2.00	2.00
Eyebrows +beard	3.00	3.00
Scalp + eyebrows	3.00	3.00
Total	100.00	100.00

Figure 5: Pie chart depicting the sites of involvement of alopecia areata (n=100)



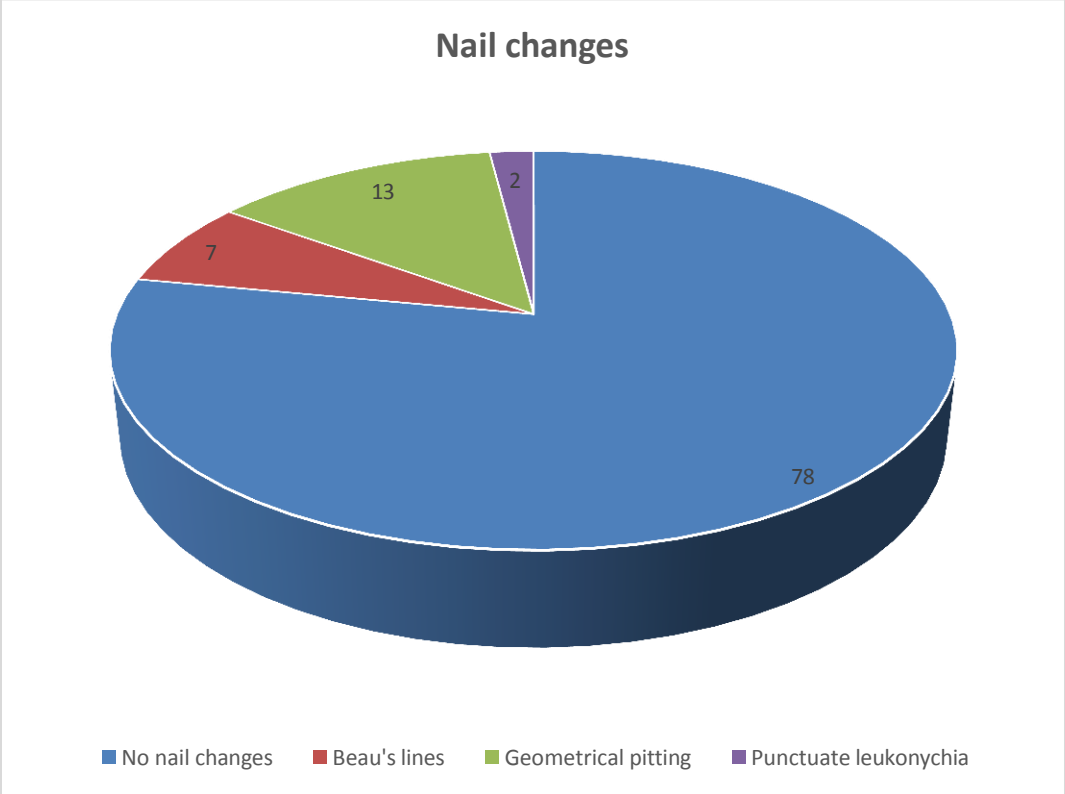
6. Nail changes in alopecia areata

Nail changes were observed in 22% of patients with alopecia areata. Beau's lines were present in 7% of cases, geometric pitting in 13%, and punctate leukonychia in only 2% of cases (table 6; figure 6).

Table 6: Nail changes among cases of alopecia areata. (n=100)

	Number of cases (%)
No nail changes	78 (78%)
Beau's lines	7 (7%)
Geometrical pitting	13 (13%)
Punctuate leukonychia	2 (2%)
Total	100 (100%)

Figure 6: Pie chart depicting the nail changes in alopecia areata. (n=100)



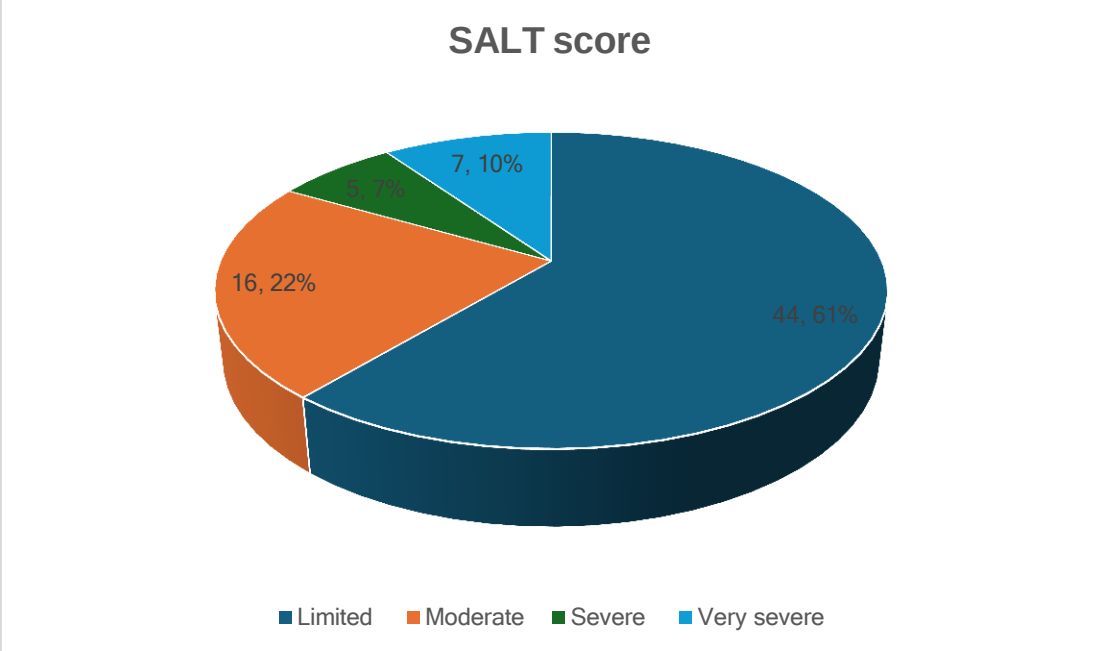
7. SALT score

Scalp involvement was present in 72 patients, and the SALT score for these patients showed that the disease was limited in 61% (n=44) of cases, moderate in 22.22% (n=16) of cases, severe in 6.94% (n=5) of cases, and very severe in 9.72% (n=7) of cases (table 7, figure 7).

Table 7: SALT score of the cases with scalp involvement (n=72)

SALT SCORE	Number of cases	Percentage
Limited	44.00	61.11
Moderate	16.00	22.22
Severe	5.00	6.94
Very severe	7.00	9.72
Total	72.00	100.00

Figure 7: Pie chart depicting the SALT score of the cases with scalp involvement (n=72)



8. Vitamin D levels comparison between age and gender

The majority of patients with alopecia areata and vitamin D deficiency were in the 21-30 age group (n=29; 50%), followed by the 31-40 age group (n=11; 19%). The distribution was similar in the control group. There was no significant difference in the age distribution of vitamin D deficiency/insufficiency between cases and controls (p-value = 0.138) (table 8, figure 8). No significant difference was seen between gender with respect to vitamin D deficiency (p value= .63) (Table 9, figure 9)

Table 8: Comparison of age group with vitamin D deficiency, insufficiency, and sufficient vitamin D in cases (n=100)

Age group	Vitamin D						Chi-square	p- value
	Deficiency(n=58)		Insufficiency(n=21)		Sufficient (n=21)			
	N	%	n	%	n	%		
≤20	5	8.60%	3	14.30%	0	0.00%		
21-30	29	50.00%	7	33.30%	10	47.60%		
31-40	11	19.00%	8	38.10%	6	28.60%		
41-50	9	15.50%	2	9.50%	1	4.80%	17.305	0.138
51-60	2	3.40%	0	0.00%	3	14.30%		
61-70	2	3.40%	1	4.80%	0	0.00%		
>70	0	0.00%	0	0.00%	1	4.80%		
	58	100.00%	21	100.00%	21	100.00%		

Figure 8: Bar chart depicting the comparison of age group with vitamin D deficiency, insufficiency, and sufficient vitamin D(n=100)

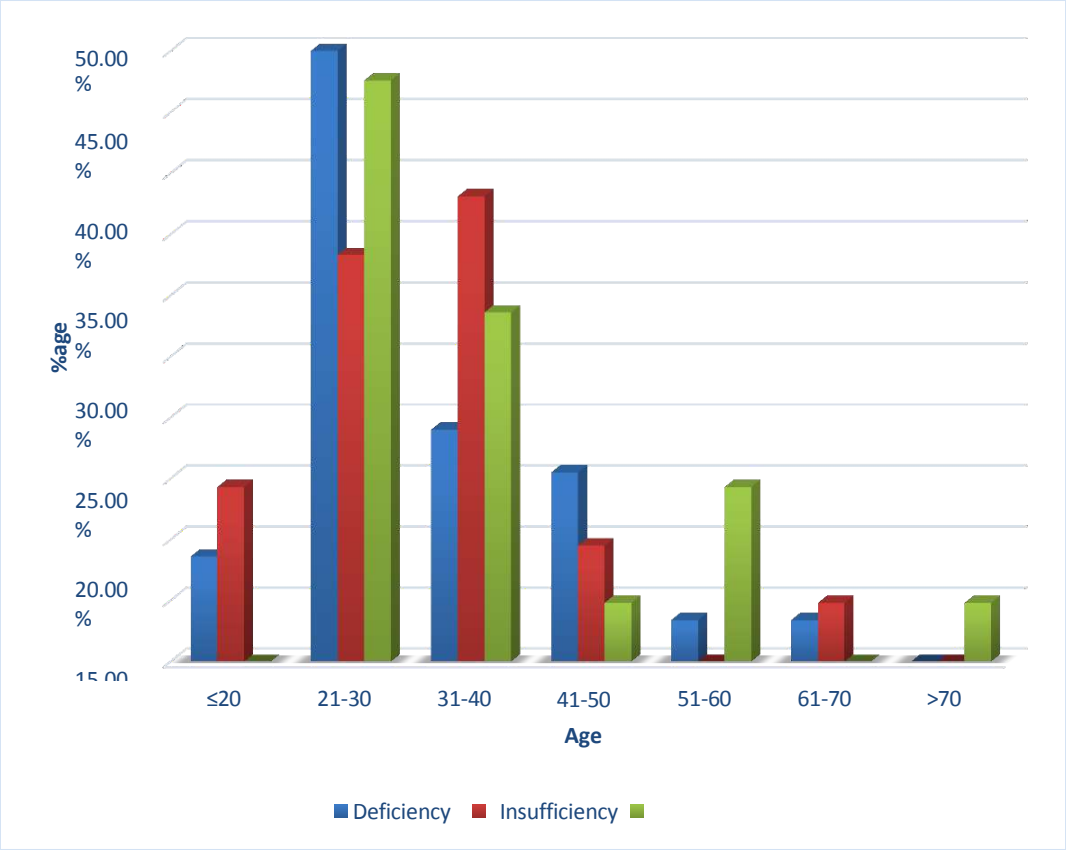
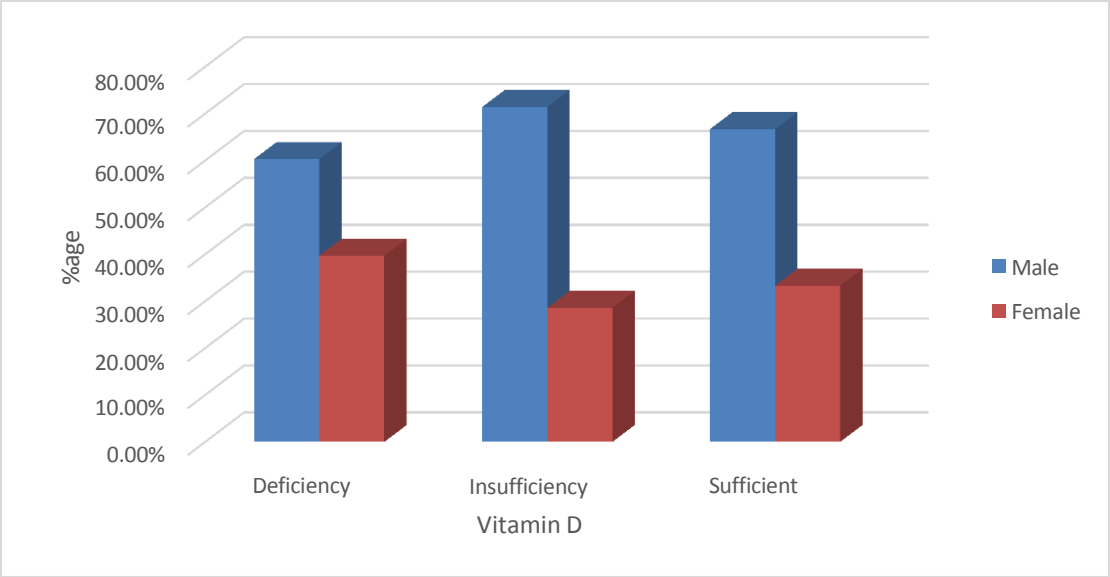


Table 9: Comparison of gender with vitamin D deficiency, insufficiency and sufficient vitamin D levels (n=100)

Gender	Vitamin D						chi-square	p-value
	Deficiency(n=58)		Insufficiency (n=21)		Sufficient (n=21)			
	n	%	n	%	n	%		
Male	35	60.30%	15	71.40%	14	66.70%	0.94	0.63
Female	23	39.70%	6	28.60%	7	33.30%		
	58	100.00%	21	100.00%	21	100.00%		

Figure 9: Bar graph depicting the comparison of gender with vitamin D deficiency, insufficiency and sufficient vitamin D levels (n=100)



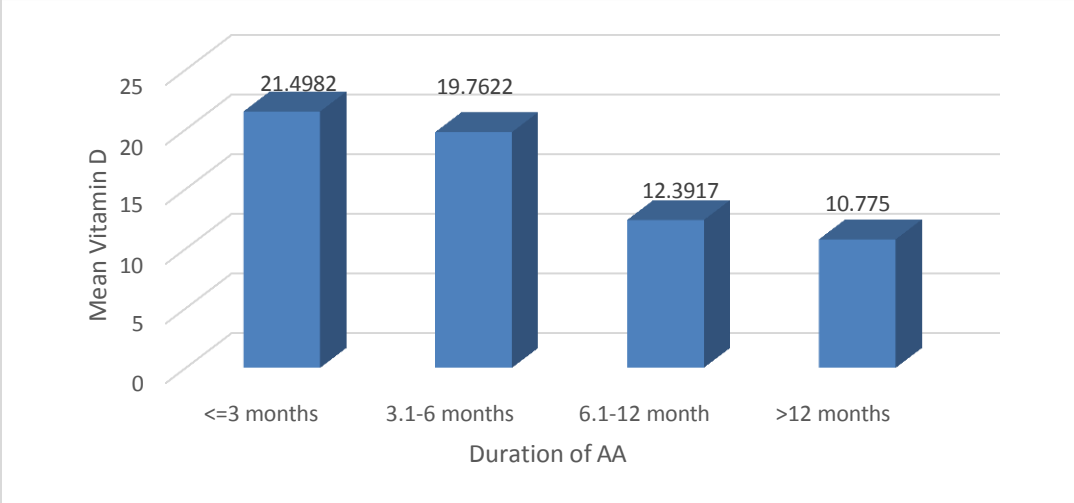
9. Vitamin D levels and duration of disease

The mean Vitamin D levels were assessed in relation to the duration of alopecia areata. Patients with a history of the disease for three months or less had mean Vitamin D levels of 21.49 ± 11.27 ng/ml. Those with a history of 3 to 6 months had a mean of 19.76 ± 9.8 ng/ml, patients with 6.1 to 12 months had a mean of 12.39 ± 6.4 ng/ml, and those with over 12 months had the lowest mean of 10.77 ± 0.31 ng/ml. We observed that as the duration of alopecia areata increased, the levels of Vitamin D tended to decrease; however, these results were statistically non-significant (p value= .132) (Table10, figure 10).

Table 10: Mean of vitamin D levels with respect to disease duration of alopecia areata(n=100)

Duration	N	Mean Vitamin D (ng/ml)	Std. Deviation	F value	p-value
<=3 months	74	21.4982	11.27162	1.916	.132
3.1-6 months	18	19.7622	9.84933		
6.1-12 month	6	12.3917	6.47174		
>12 months	2	10.7750	0.31820		

Figure 10: Mean of vitamin D levels with respect to disease duration of alopecia areata(n=100)



10. Vitamin D levels and site of alopecia areata

The mean Vitamin D levels were highest in patients with alopecia areata affecting the eyebrows, with a mean of 23.27 ± 9.73 ng/ml. This was followed by hair loss at the beard, with a mean Vitamin D level of 22.82 ± 21 ng/ml, and scalp involvement, with a mean Vitamin D level of 19.6 ± 10.54 ng/ml. The lowest mean Vitamin D levels were observed in patients with whole-body involvement, at 11.90 ± 5 ng/ml (Table 11a, figure 11a). The interpretation of Vitamin D levels as deficient, insufficient, and sufficient showed no statistical significance when compared to patients with alopecia areata at different body sites (p value= .757) (Table 11b, figure 11b).

Table 11a: Mean Vitamin D levels with respect to body site of alopecia areata (n=100)

Site of AA	Number	Mean Vitamin D (ng/ml)	Std. Deviation	p-value
Scalp	61.00	19.79	10.91	0.382
Beard	16.00	23.94	9.92	
Eyebrows	10.00	23.51	14.22	
Whole body	5.00	11.90	5.47	
Scalp+ beard	2.00	14.36	0.75	
Eyebrows +beard	3.00	22.47	15.15	
Scalp eyebrows	3.00	20.47	3.27	
Total	100.00	20.42	10.92	

Figure 11a : Bar graph depicting the mean Vitamin D levels with respect to body site of alopecia areata (n=100)

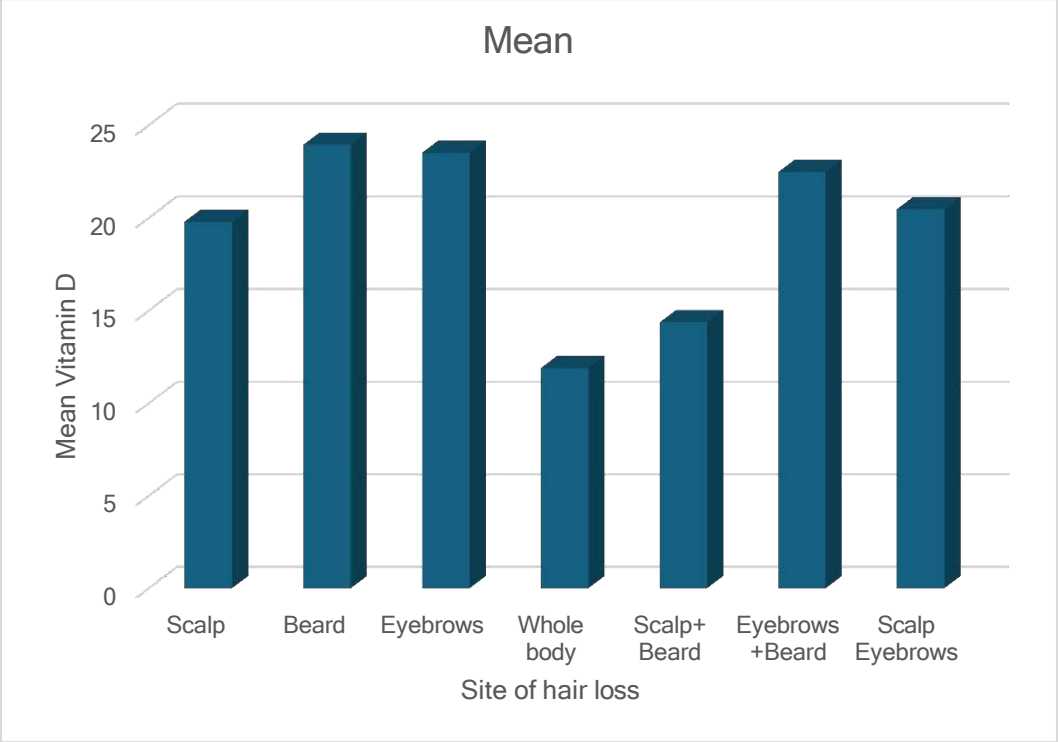
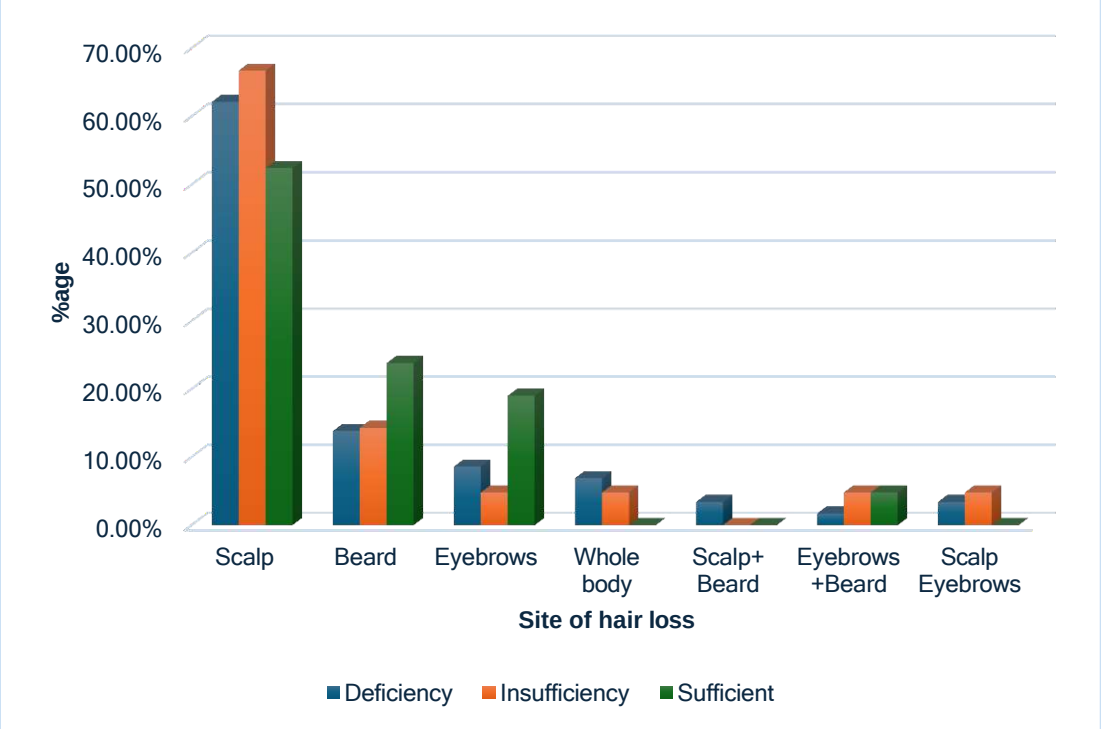


Table 11 b: Comparison of site of hair loss with vitamin D Deficiency, Insufficiency, and Sufficient vitamin D levels. (n=100)

Site of hair loss	Vitamin D						chi-square	p-value
	Deficiency(n=58)		Insufficiency(n=21)		Sufficient(n=21)			
	n	%	n	%	n	%		
Scalp	36	62.10%	14	66.70%	11	52.40%	8.352	.757
Beard	8	13.80%	3	14.30%	5	23.80%		
Eyebrow	5	8.60%	1	4.80%	4	19.00%		
Whole Body	4	6.90%	1	4.80%	0	0.00%		
Scalp+ Beard	2	3.40%	0	0.00%	0	0.00%		
Eyebrows+Beard	1	1.70%	1	4.80%	1	4.80%		
Scalp + Eyebrow	2	3.40%	1	4.80%	0	0.00%		
Total	58	100.0%	21	100.00%	21	100.00%		

Figure 11b: Bar graph comparing of site of hair loss with vitamin D Deficiency, Insufficiency, and Sufficient vitamin D levels. (n=100)



11. Vitamin D levels and pattern of alopecia areata

When assessing the diagnosis of Vitamin D levels and the pattern of alopecia areata, there was no statistical significance (p-value = .156). However, all patients with sufficient Vitamin D levels had patchy alopecia areata (AA).None of the patients with alopecia totalis, alopecia universalis, or diffuse alopecia areata had sufficient Vitamin D levels. Among the 58 patients with Vitamin D deficiency, 48 (82.8%) had patchy AA, 3 (5.2%) had alopecia totalis, 4 (6.9%) had alopecia universalis, and 3 (5.3%) had diffuse AA (Table 12a, figure 12a).

However, the comparison of mean Vitamin D levels with the pattern of alopecia areata was statistically significant (p-value = .038). Patients with patchy alopecia areata had the highest mean Vitamin D levels at 21.38 ±

11.02 ng/ml, followed by those with diffuse alopecia areata at 19.78 ± 8.30 ng/ml. Patients with alopecia universalis and alopecia totalis had lower mean Vitamin D levels at 9.67 ± 1.48 ng/ml and 8.27 ± 2.57 ng/ml, respectively. These results indicate a statistically significant difference in Vitamin D levels based on the pattern of alopecia areata (Table 12b, figure 12b).

Table 12a: Comparison of Pattern of hair loss with vitamin D Deficiency, Insufficiency and Sufficient vitamin D

Pattern of hair loss	**Vitamin D**						**chi-square**	**p-value**
	Deficiency(n=58)		**Insufficiency(n=21)**		**sufficient (n=21)**			
	n	%	n	%	N	%		
Patchy AA	48	82.80%	18	85.70%	21	100.00%	9.330	.156
Alopecia Totalis	3	5.20%	0	0.00%	0	0.00%		
Alopecia Universalis	4	6.90%	0	0.00%	0	0.00%		
Diffuse AA	3	5.20%	3	14.30%	0	0.00%		
Total	58	100.00%	21	100.0%	21	100.00%		

Figure 12a: Bar chart depicting the comparison of Pattern of hair loss with vitamin D deficiency, insufficiency and sufficient vitamin D (n=100)

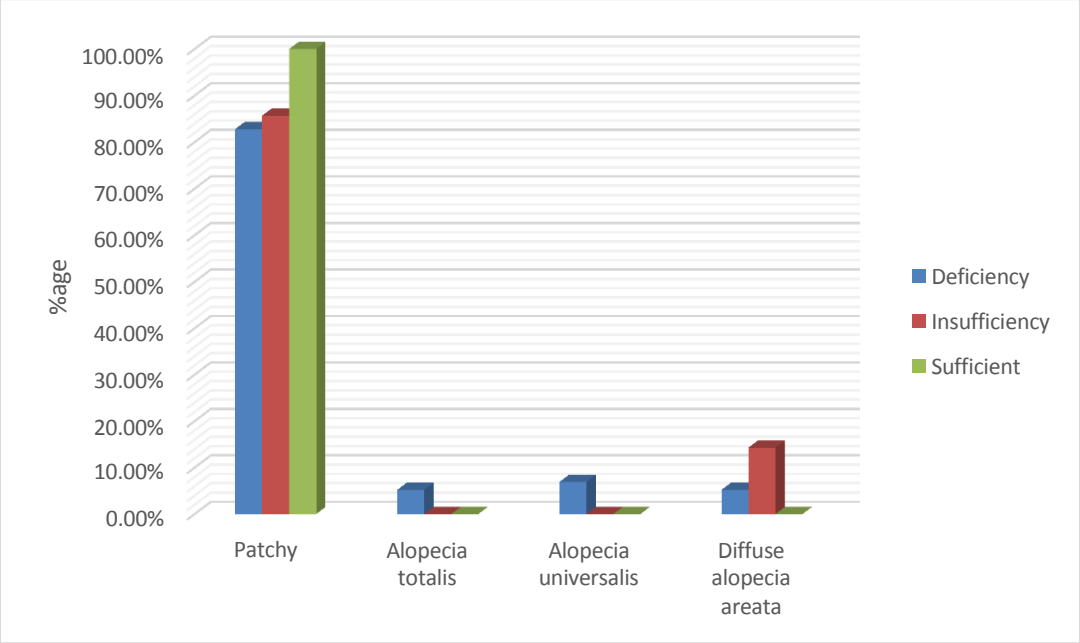
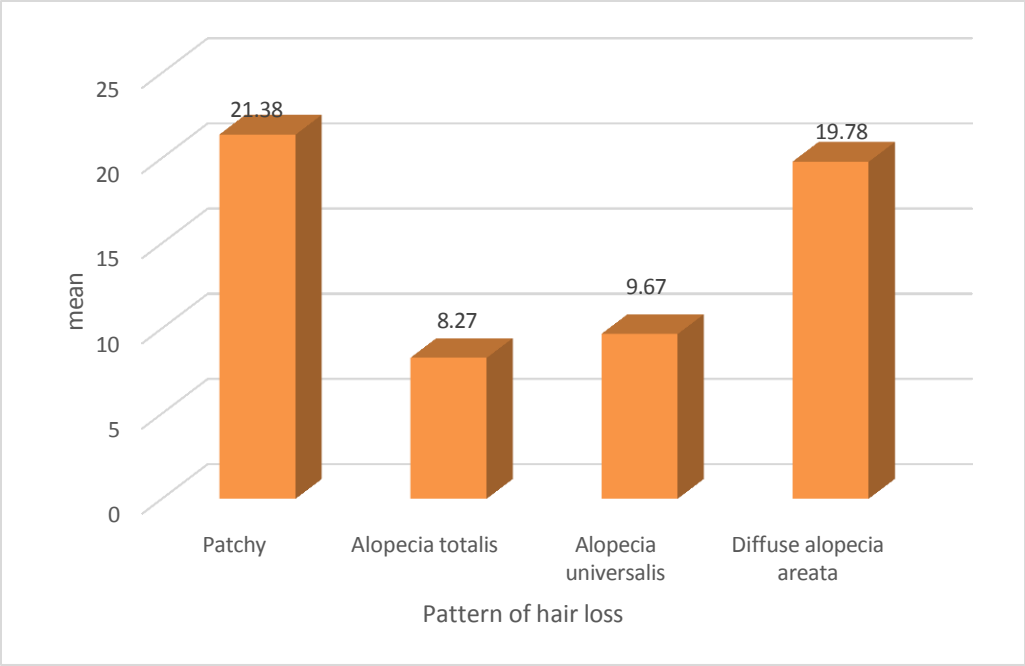


Table 12b: Mean vitamin D levels with respect to patterns of alopecia areata. (n=100)

Pattern of Alopecia Areata	N	Mean Vitamin D (ng/ml)	Std. Deviation	p-value
Patchy AA	87.00	21.38	11.02	0.038
Alopecia Totalis	3.00	8.27	2.57	
Alopecia Universalis	4.00	9.67	1.48	
Diffuse AA	6.00	19.78	8.30	
Total	100.00	20.42	10.92	

Figure 12b: Bar chart depicting the mean vitamin D levels with respect to patterns of alopecia areata. (n=100)



12. Vitamin D and nail changes

Among the 22 patients who had nail changes, 16 (27.6%) had a deficiency of Vitamin D, while 5 (23.8%) had insufficient Vitamin D levels (Table 13a, figurea). The mean Vitamin D level in patients with nail changes was 16.01 ± 8.91 ng/ml, significantly lower than patients who did not have nail changes, with a mean of 21.66 ± 11.15 ng/ml (p-value: .03) (Table13b, figure 13b).

Table 13a: Comparison of nail changes with vitamin D deficiency, insufficiency and sufficient vitamin D

Nail changes	Vitamin D						chi-square	p-value
	Deficiency (n=58)		Insufficiency (n=21)		Sufficient (n=21)			
	n	%	n	%	n	%		
Absent	42	72.40%	16	76.20%	20	95.20%	4.731	.094
Present	16	27.60%	5	23.80%	1	4.80%		
Total	58	100.00%	21	100.00%	21	100.00%		

Figure 13a: Bar graph depicting the comparison of nail changes with vitamin D deficiency, insufficiency and sufficient vitamin D

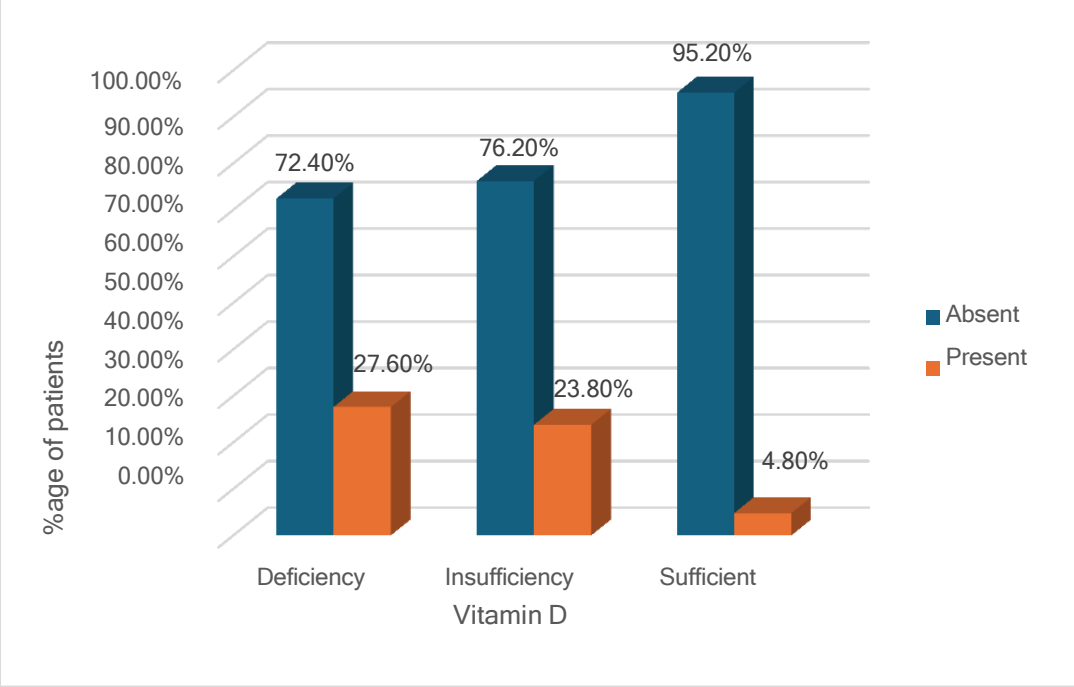
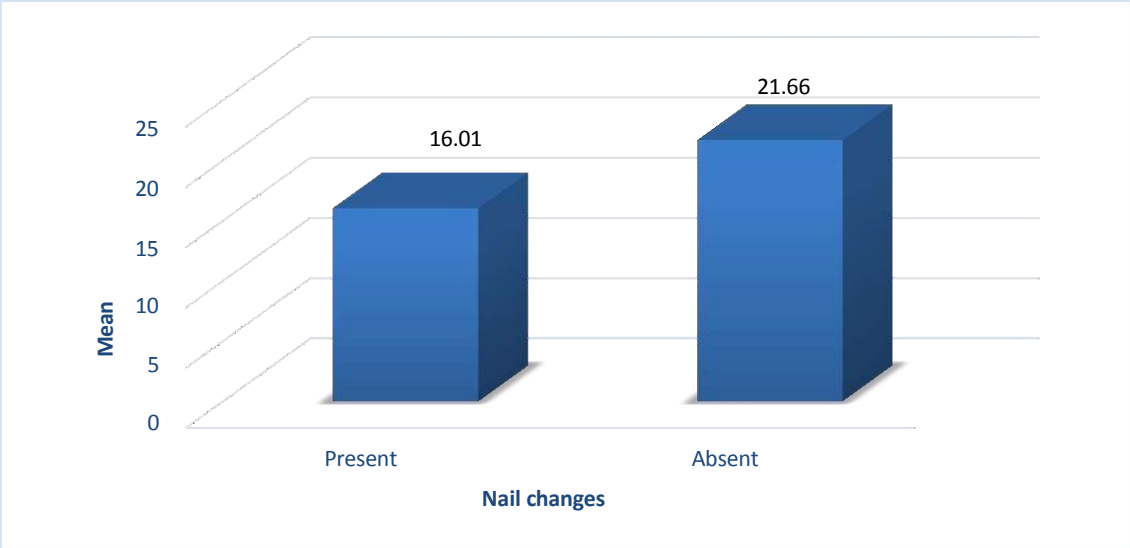


Table 13b: Mean of vitamin D for presence of nail changes (n=100)

Nail changes	n	Mean Vitamin D (ng/ml)	SD	t-value	p-value
Present	22	16.01	8.91	-2.183	.03
Absent	78	21.66	11.15		

Figure 13b: bar graph depicting the mean of vitamin D for presence of nail changes (n=100)



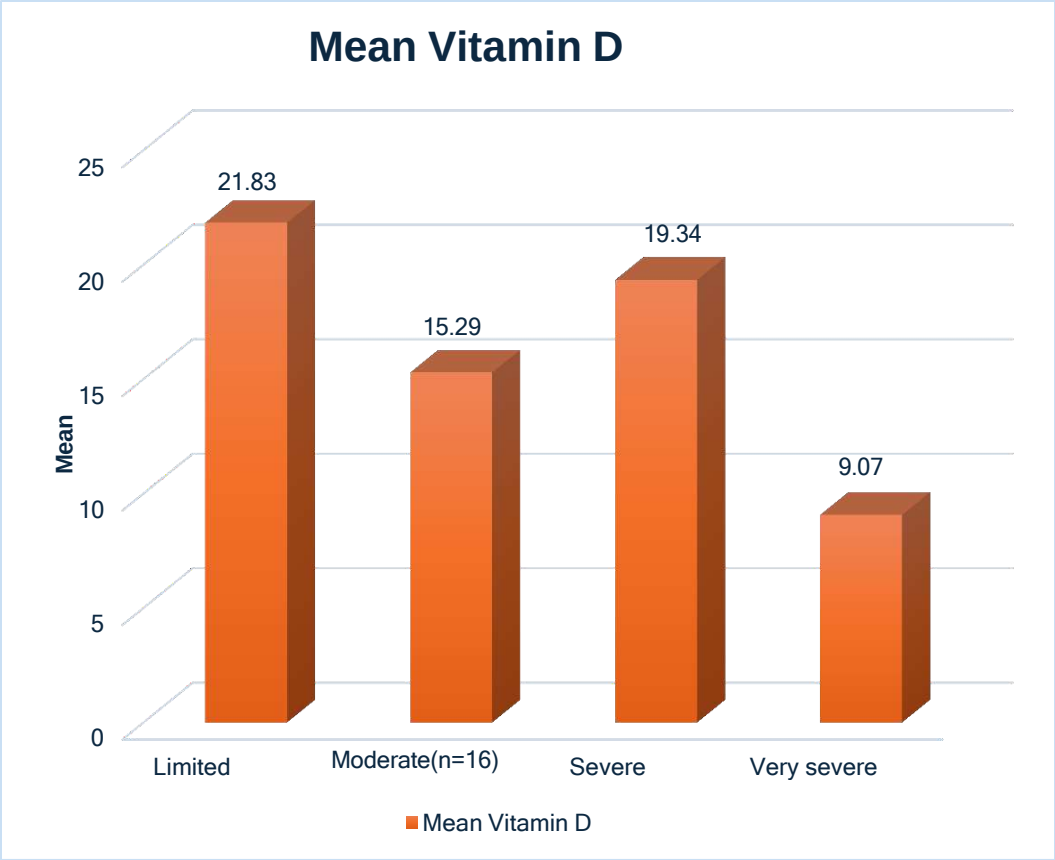
13. Vitamin D and SALT score

The SALT score was limited in 44 cases, with a mean Vitamin D level of 21.83 ± 11.26 ng/ml. In moderate cases (n=16), the mean Vitamin D level was 15.29 ± 7.45 ng/ml, while in severe cases (n=5), it was 19.34 ± 6.18 ng/ml. Very severe cases (n=7) had a mean Vitamin D level of 9.07 ± 1.97 ng/ml (Table 14, figure 14). This association was found to be statistically significant (p value- .007). A mild inverse correlation was observed between SALT scoring and Vitamin D levels, implying that as the SALT score increases, Vitamin D levels mildly decrease. This correlation was statistically significant (r = -0.366; p = 0.002).

Table 14: Mean Vitamin D levels as compared to SALT scoring (n=100)

	N	Mean Vitamin D (ng/ml)	Std. Deviation	p-value
Limited	44.00	21.83	11.26	0.007
Moderate	16.00	15.29	7.45	
Severe	5.00	19.34	6.18	
Very severe	7.00	9.07	1.97	
Total	72.00	18.96	10.43	

Figure 14: Bar graph depicting the mean Vitamin D levels as compared to SALT scoring(n=100)



DISCUSSION

Alopecia Areata (AA), a chronic inflammatory disorder characterized by reversible non-cicatricial hair loss, affects approximately 2% of the general population at some point in their lifetime.[2] As a T-cell driven autoimmune condition, AA can present in various forms, ranging from a single spot of hair loss to multiple patches, complete scalp baldness, or widespread hair loss across the body.[9,24,59] The impact on physical appearance often leads to psychiatric comorbidities, significantly affecting patients' quality of life by diminishing their emotional and social well-being.[23] Research indicates that significant depression occurs in 8.8% of AA patients over their lifetime.[28] Vitamin D has been proposed as a risk factor for AA due to its immunomodulatory properties.[106] Studies suggest that Vitamin D and its analogs not only help prevent the development of autoimmune diseases but may also serve as treatment options.[22] Vitamin D is integral to the pathogenesis of AA, with 1,25(OH)2D3 playing a crucial role in hair follicle biology. The expression of the Vitamin D receptor (VDR) is essential for normal hair follicle development and cycling.[70,107,108] This study aims to evaluate the difference in Vitamin D levels between AA patients and healthy controls, and to assess the impact of Vitamin D on the severity of the disease.

The literature reviews estimate the mean age of onset for AA to be between 25 and 36 years. The mean age of presentation for AA in our study was 33.51±11.49 years. This finding is consistent with previous reports, which indicate that the majority of AA patients experience their first onset before the age of 40. In a Danish cohort, The mean age of AA onset was 32.7±17.6 years.[109] The majority of our patients fell within the 21–30-year age range, consistent with findings by Bhat et al.[16]

Previous epidemiological studies have shown conflicting results regarding the gender distribution among AA patients, with some studies reporting equal distribution and others indicating a predominance of one gender. These variations can be attributed to differences in study populations and designs. In our study, there was no significant difference in gender distribution between cases and controls. However, the majority of patients in our study were male.

This study primarily investigates vitamin D levels in patients with alopecia areata (AA) compared to healthy controls. Hair loss in AA is attributed to disruptions in the hair follicle (HF) cycle. The precise mechanism linking vitamin D deficiency to AA remains unclear, but several key elements of the autoimmune process in AA are believed to be involved, including the loss of immune privilege in hair follicles, autoreactive effector T cells and mast cells, cytotoxic T cells, the JAK/STAT pathway, and effects on T regulatory cells (Tregs) and oxidative stress. Vitamin D receptors (VDRs) play a crucial role in hair cycling.[16,22,72,110]

The expression of VDRs and the activating enzyme 1α-hydroxylase on various immune cells, such as macrophages, dendritic cells, and T cells, indicates that vitamin D influences both the innate and acquired immune systems. VDR is a ligand-inducible transcription factor regulating vitamin D-responsive genes and belongs to the nuclear hormone receptor family. Vitamin D may help maintain the immune privilege of hair follicles by reducing the production of interferon-gamma (IFN-γ).[69,83]

Numerous studies have shown that 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) modulates dendritic cell activity, suppressing their differentiation and maturation, as well as the expression of MHC Class II, CD40, CD80, and CD86. It also inhibits the secretion of proinflammatory cytokines, including interleukins (IL-1, IL-2, IL-6, IL-12), IFN-γ, and tumor

necrosis factor-alpha (TNF-α).[106] Additionally, 1,25(OH)2D3 inhibits IL-12, which is involved in shifting the immune response from a Th1 to a Th2 phenotype, thus helping to suppress Th1-mediated autoimmune responses and promoting Th2 responses. Moreover, 1,25(OH)2D3 enhances the production and functionality of T-regulatory cells, contributing further to the suppression of autoimmune disorders.[81,108] The collapse of HF immune privilege and the exposure of autoantigens lead to the accumulation of autoreactive effector T cells. Decreased VDR expression in AA is thought to disrupt HF cycling due to an autoimmune response. Furthermore, reactive oxygen species (ROS) play a role in the pathophysiology of AA, and vitamin D has been demonstrated to have antioxidant properties in several clinical trials.[111–113]

This study revealed a significant difference in Vitamin D levels between the case and control groups (p-value <.001). Vitamin D deficiency (levels less than 20 ng/ml) was observed in 58% of patients in the case group compared to 28% in the control group. Vitamin D insufficiency (20-40 ng/ml) was found in 21% of patients in the case group and 21.30% in the control group (Table 3a, Figure 3b). The mean Vitamin D levels in the case group were significantly lower, at 20.42 ± 10.92 ng/ml, compared to the control group, which had mean levels of 30.09 ± 16.12 ng/ml (p-value <

.001) (Table 3b, Figure 3b). A study by Rehman et al found similar results: in the case group, a significant proportion of patients (37.78%) had deficient serum Vitamin D levels, followed by 34.81% with insufficient levels.[114] In contrast, the control group had a higher proportion of patients with normal serum Vitamin D levels (42.22%) and a smaller proportion with deficient levels (23.70%). These findings underscore the association between lower Vitamin D levels and alopecia areata. The Vitamin D levels for both groups are detailed in Table 3.

Table 15: Various studies assessing the serum vitamin D levels in patients of Alopecia areata

Study	Year	Vitamin D levels (mean) in cases	No.of Cases (n)	Vitamin D Levels (mean) in controls	No. of Controls (n)	Significance
Our study	2024	20.42 ng/ml	n=100	30.09 ng/ml	n=50	Significant
Gupta et al[115]	2023	17.15 ng/ml	n=25	34.58 ng/ml	n=25	Significant
Darwishet al[116]	2017	7.52 ± 6.24 ng/ml	n=30	31.70 ± 12.29 ng/ml	n=30	Significant
Bhat et al[16]	2017	16.6 ng/ml	n=50	25.49 ng/ml	n=35	Significant
Rehman et al[114]	2019	22.87ng/ml	n=135	27.34 ng/ml	n=135	Significant

The duration of alopecia areata (AA) varies significantly among patients, as reported in various studies. In our study, the mean duration of AA was 3.98 ± 6.98 months. This broad range highlights the variability in disease duration among the patients. A detailed analysis showed that 74% of patients had a history of AA for three months or less, 18% had the disease for 3.1 to 6 months, 6% had it for 6.1 to 12 months, and only 2% had a duration of more than 12 months. Comparing these results with other studies provides a clearer perspective on the variability of AA duration. In the study by Gupta et al., the majority of patients (56%) had AA for six months or less, reflecting a relatively shorter duration similar to our findings.[115] On the other hand, Rehman et al. reported a wider range of disease duration, from 10 days to 20 years, with a mean duration of 1.32 ±2.44 years.[114] However, they observed that the maximum number of cases had a disease duration of 3–12 months (49.63%), followed by <3 months (26.66%), suggesting that most patients experience the disease for less than a year. The study by Bhat et al. indicated a mean disease duration of 4.80 ± 4.58 months, slightly higher than our findings but still within a similar range.[16] This reinforces the notion that AA duration can vary but often falls within a few months for many patients. These

variations in disease duration can be attributed to several factors, including differences in study populations and geographical variations. The chronic and recurrent nature of AA might lead to fluctuations in the perceived duration of the disease.

Patients might experience episodes of hair loss and regrowth, complicating the accurate measurement of disease duration. When assessing the relationship between the duration of alopecia areata (AA) and vitamin D levels, an inverse trend was observed: as the duration of AA increased, vitamin D levels tended to decrease. However, this observed trend did not reach statistical significance ($p = 0.132$), as depicted in Table 10.

The distribution of alopecia areata (AA) patches in our study showed significant variability, with the majority of cases (61%) exhibiting patches exclusively on the scalp. This finding is consistent with previous studies. For instance, Dasankunju et al. reported that 93.3% of their 30 patients had scalp involvement.[117] Similarly, Patil et al found scalp involvement in 87% of their 100 AA patients, aligning with our observation that the scalp is the most affected site.[118] Siddappa et al.'s study on 100 AA patients further supports this, showing that 63% had scalp involvement, 24% had beard involvement, 8% had both scalp and beard involvement, 4% had scalp and eyebrow involvement, and 1% had involvement of the scalp, beard, and eyebrows.[119] Rehman et al. also reported high scalp involvement, affecting 90.37% of their 135 cases, with the beard affected in 28.89%, eyebrows in 9.63%, and other body sites in 7.41%.[114]

Our findings also revealed that mean vitamin D levels varied according to the affected body site. Patients with eyebrow involvement had the highest mean vitamin D levels at 23.27 ± 9.73 ng/ml, followed by those with beard involvement at 22.82 ± 21 ng/ml, and scalp involvement at 19.6 ± 10.54 ng/ml. The lowest levels were observed in patients with whole-body involvement at 11.90 ± 5 ng/ml. The comparison of mean Vitamin D levels

with the pattern of alopecia areata was also statistically significant (p-value = .038). Patients with patchy alopecia areata had the highest mean Vitamin D levels at 21.38 ± 11.02 ng/ml, followed by those with diffuse alopecia areata at 19.78 ± 8.30 ng/ml. Patients with alopecia universalis and alopecia totalis had lower mean Vitamin D levels at 9.67 ± 1.48 ng/ml and 8.27 ± 2.57 ng/ml, respectively. Rehman et al. found similar trends, with mean vitamin D levels of 21.71 ± 6.68 ng/ml in patients with patchy AA involving both the scalp and beard, 23.75 ± 11.01 ng/ml in patients with only scalp involvement, and 28.73 ± 6.91 ng/ml in patients with only beard involvement.[114] However, the differences were not statistically significant (P = 0.13), mirroring our findings where the relationship between AA duration and vitamin D levels was also not statistically significant (P = 0.132). The results of the study conducted by Gupta et al. showed that there was an insignificant variation in serum vitamin D along with the clinical pattern of the disease.[115]

The levels showed a gradual decline as the disease pattern progressed from patchy AA to alopecia universalis, with mean values of 17.63 ng/ml and 12.305 ng/ml, respectively. However, no significant correlation was found between vitamin D levels and the AA pattern (P=0.075), which may be attributed to the small sample size. Our study confirms that the scalp is the most commonly affected site in AA, consistent with previous research. While there is a trend suggesting lower vitamin D levels with more extensive AA involvement, this relationship was not statistically significant.

The present study showed the same pattern of decline. However, in this study, the significant correlation was consistent with the findings reported by D'Ovidio et al. and Mahamid et al. Bakry et al. also found a significant correlation between vitamin D levels and AA patterns.[72,73,77] In a study by Bhatt et al., the patients with single-patch alopecia had a mean concentration of 25(OH)D of 19.9 ± 4.45 ng/ml whereas those with

multiple patches had a mean value of 12.0 ± 4.45 ng/ml and the difference was statistically significant (P < 0.001).[16] A significant negative correlation was found between number of patches and Vitamin D level (P < 0.001, r =−0.670). Furthermore, in the study by Rehman et al. the mean serum Vitamin D level progressively decreased from the patchy pattern (23.95 ± 9.78 ng/mL), ophiasis pattern (23.83 ± 10.44 ng/mL), and reticular pattern (18.55 ± 8.44 ng/mL) to complete baldness pattern (11.24 ± 5.76 ng/mL). The results were observed to be highly significant (P = 0.004).[114]

In the present study, we observed no significant difference between genders concerning vitamin D deficiency among cases (p = 0.63) (Table 9). This finding aligns with the results of previous studies which found no difference in vitamin D levels concerning age and gender, consistent with our observations.[15] However, our findings contrast with those of Gupta et al. who reported that males had relatively higher serum vitamin D values compared to females.[115] This discrepancy highlights the need for further investigation into potential gender-related differences in vitamin D levels among patients with AA.

Nail changes were observed in 22% of patients with AA in our study. Specifically, Beau's lines were present in 7% of cases, geometric pitting in 13%, and punctate leukonychia in 2% (Table 6). This incidence aligns with previous studies. Dasankunju et al. Reported nail pitting in 30% of their patients, while Patil et al. found nail changes in 21% of their patients, with pitting being the most common change observed in 10 patients.[117,118] Additional nail changes noted by Patil et al. included longitudinal ridges in 5 patients and leukonychia and lusterless nails in 3 patients each.[118] Similarly, Gupta et al. observed nail changes in 32% of their 30 patients.[115] In contrast, Rehman et al. reported a lower incidence of nail changes, found in 11.11% of their 135 patients.[114] Interestingly they also reported that the serum level of 25(OH)-Vitamin D was

significantly lower in patients with nail involvement (17.72 ± 7.41 ng/ml) compared to those without nail involvement (23.52 ± 10.15 ng/ml), with a statistically significant difference (P = 0.03). This finding was consistent with our study, where the mean vitamin D level in patients with nail changes was 16.01 ± 8.91 ng/ml, significantly lower than the 21.66 ± 11.15 ng/ml observed in patients without nail changes (P = 0.03) (Table 13). Nassiri et al. also reported significantly lower serum levels of 25(OH)-Vitamin D in patients with nail involvement compared to those without.[74] However, Bakry et al. found no significant association between 25(OH)-Vitamin D levels and nail involvement, highlighting some inconsistency in the literature.[73] These studies and findings in our studies suggest that nail changes are a common manifestation in AA and are associated with lower vitamin D levels.

Severity of Alopecia Tool (SALT) scoring for these patients revealed that the disease was limited in 61% (n=44) of cases, moderate in 22.22% (n=16) of cases, severe in 6.94% (n=5) of cases, and very severe in 9.72% (n=7) of cases (Table 7, Figure 7). This distribution is comparable to the findings of Rehman et al., who reported that the majority of their patients had S1 grade (42.62%), followed by S2 grade (28.69%), S3 grade (13.93%), S4 grade (9%), and S5 grade (5.74%).[114] Similarly, Yilmaz et al. found that 71.4% of their cases were in S1 grade, 14.2% in S2, 7.4% in S3, 4.7% in S4, and 2.3% in S5 grade, which is in accordance with our study. In our study, the SALT score was associated with varying mean vitamin D levels.[80] Limited cases (n=44) had a mean vitamin D level of 21.83 ± 11.26 ng/ml. Moderate cases (n=16) had a mean vitamin D level of 15.29 ± 7.45 ng/ml, severe cases (n=5) had a mean vitamin D level of 19.34 ± 6.18 ng/ml, and very severe cases (n=7) had a mean vitamin D level of 9.07 ± 1.97 ng/ml (Table 14, Figure 14). This association was statistically significant (p = 0.007). We observed a mild inverse correlation between SALT scoring and vitamin D levels, indicating that as the SALT score increases, vitamin D levels mildly

decrease. This correlation was statistically significant (r = -0.366, p = 0.002). The results of our study are consistent with those of Rehman et al., who also found a significant association between SALT scores and mean serum vitamin D levels (P < 0.000). They reported that patients with higher SALT grades (S4 & S5) had extremely low serum vitamin D levels, resulting in a significant negative correlation between the severity of AA and vitamin D levels (P = 0.00, r = -0.474).[114] Bhat et al. also reported a significant negative correlation between SALT scores and vitamin D levels. In their study, patients with an S1 score had a mean vitamin D concentration of 19.02 ± 4.4 ng/ml, while those with an S2 score had a mean concentration of 9.00 ± 2.80 ng/ml (P< 0.001; r = -0.730), which is consistent with our findings.[16] These findings collectively suggest that lower vitamin D levels are associated with higher SALT scores, indicating more severe forms of AA. This significant negative correlation highlights the potential role of vitamin D deficiency in the progression and severity of AA.

Given the role of vitamin D in the pathogenesis of alopecia areata (AA), it may be considered as a potential treatment for the disease. Experimental autoimmune models have demonstrated the beneficial effects of $1,25(OH)_2D_3$ supplementation, but the systemic use of vitamin D in treating human autoimmune diseases remains under investigation. In clinical applications, the supraphysiological doses of active vitamin D required to modulate immune responses may lead to calcemic side effects. To mitigate this issue, hypocalcemic analogs of active vitamin D with similar immunoregulatory activities are being explored. One such analog is calcipotriol, a vitamin D3 analog, which is at least 100 times less calcemic than calcitriol. Recent studies have shown promising results with the use of calcipotriol in AA treatment.[110]

For instance, Narang et al. conducted a prospective study involving 22 AA patients treated with 0.005% calcipotriol lotion twice daily for three months. After 12 weeks of treatment, hair regrowth was observed in 13

(59.1%) patients. The mean period for disease stabilization and hair regrowth was 4 weeks and 4.21 ± 2.13 weeks, respectively. The response to treatment was significantly better in patients with lower baseline vitamin D levels. The authors concluded that topical calcipotriol could be an alternative treatment for AA. These findings suggest that vitamin D and its analogs, particularly in topical formulations, offer a promising therapeutic avenue for AA.[63]

Our study findings suggest a potential link between vitamin D levels and the pathogenesis of alopecia areata (AA). The observed lower levels of 25(OH)D in AA patients compared to healthy controls, along with the negative correlation between 25(OH)D levels and the severity of AA assessed by the Severity of Alopecia Tool (SALT) score, indicate a possible role of vitamin D in the development and progression of AA. Specifically, patients with lower vitamin D levels tended to have more severe AA, as reflected in higher SALT scores. This inverse relationship between vitamin D levels and disease severity underscores the potential importance of vitamin D in modulating the course of AA. The significant association between nail changes and the pattern of the disease further strengthens the hypothesis of vitamin D involvement in AA. Patients with nail changes, a common manifestation in AA, exhibited significantly lower vitamin D levels compared to those without nail involvement. This association provides additional evidence supporting the role of vitamin D in AA and suggests that vitamin D deficiency might contribute to both the dermatological and nail-related symptoms of the disease. The suggestion of vitamin D supplementation as a possible treatment for AA is promising. However, further research, particularly clinical trials, is needed to validate this hypothesis and determine the optimal dosage and duration of supplementation.

Our study contributes valuable information to the understanding of AA pathophysiology and opens avenues for exploring vitamin D supplementation as a potential therapeutic intervention. The findings

highlight the need for a multidisciplinary approach to AA treatment, considering not only dermatological but also nutritional and immunological aspects. Future research should aim to elucidate the exact role of vitamin D in AA, investigate its potential as a preventive measure, and develop evidence-based guidelines for vitamin D supplementation in AA patients.

In conclusion, while our study provides compelling evidence of a link between vitamin D levels and AA, further research is essential to confirm these findings and to explore the potential therapeutic benefits of vitamin D. This could ultimately lead to improved management strategies for AA, benefiting patients with this challenging and often distressing condition. While our study provides valuable insights into the potential link between vitamin D levels and alopecia areata (AA), several limitations must be acknowledged. The relatively small sample size may limit the generalizability of our findings, and the cross-sectional design only provides a snapshot in time, preventing causal inferences. We did not control confounding factors such as dietary habits, sun exposure, and genetic predispositions which could influence both vitamin D levels and AA severity. The study relied on a single measurement of serum 25(OH)D levels, which can fluctuate. The diversity of the study population was limited, potentially affecting the applicability of the results to broader groups. The subjective nature of the SALT score for assessing AA severity introduces potential bias. Other potential biases, including selection and information bias may also have influenced the results. These limitations highlight the need for further research with larger, more diverse cohorts, longitudinal designs, and better control for confounding factors to validate and extend our findings.

SUMMARY AND CONCLUSION

SUMMARY

The present study was conducted on 100 Patients of alopecia areata of age 18 years or above attending skin and VD OPD of Dayanand Medical College & Hospital, Ludhiana constituted the study material. This study aimed to evaluate the difference in Vitamin D levels between AA patients and healthy controls and to assess the impact of Vitamin D on the severity of the disease.

- The age distribution was comparable in both cases and controls with the majority of patients between the age of 21-30 years (cases: 46,46%; controls: 13,26%) followed by 31-40 years (cases:25,25%; controls: 15,30%), 31-40 years (cases: 12,12%; controls: 9.18%) (p value= .302)
- There were 64 (64%) males and 36 (36%) females among the cases and 35 (35%) males and 15 (30%) females among the controls. The distribution of the genders was similar, with males outnumbering females in both the groups (p value=.465)
- The study revealed a significant difference in Vitamin D levels between the case and control groups (p value < .001), with Vitamin D deficiency observed in 58% of patients in the case group compared to 28% in the control group. Vitamin D insufficiency was found in 21% of patients in the case group and 21.30% in the control group. Vitamin D sufficiency was achieved in 21% of patients in the case group, whereas 30.70% of the control group exhibited sufficient levels of Vitamin D. The mean Vitamin D levels in the case group were significantly lower, at 20.42 ± 10.92 ng/ml, compared to the control group, which had mean levels of 30.09 ± 16.12 ng/ml (p-value < .001)
- The duration of the disease was variable in all the patients. 74 (74%) patients had a history of alopecia areata for three months or

less, 18% had a history of alopecia areata lasting 3.1 to 6 months, 6% had it for 6.1 to 12 months, and only 2% had alopecia areata for more than 12 months. The mean duration of alopecia areata among patients was 3.98 ± 6.98 months(Table 4; Figure 4).

- The distribution of alopecia areata patches among patients varied across different body sites. The majority of cases (61%) exhibited patches exclusively on the scalp, followed by 16% with patches solely on the beard and 10% with patches confined to the eyebrows. A smaller proportion of patients (5%) experienced patches across their entire body. Additionally, 2% of patients had patches present on both the scalp and beard, while 3% had patches on both the eyebrows and beard, and another 3% had patches on both the eyebrows and scalp. This diverse distribution highlights the variability in the presentation of alopecia areata among individuals, with certain sites being more commonly affected than others.
- Nail changes were observed in 22% of patients with alopecia areata. Beau's lines were present in 7% of cases, geometric pitting in 13%, and punctate leukonychia in only 2% of cases.
- Scalp involvement was present in 72 patients, and the SALT scoring for these patients showed that the disease was limited in 61% (n=44) of cases, moderate in 22.22% (n=16) of cases, severe in 6.94% (n=5) of cases, and very severe in 9.72% (n=7) of cases.
- The majority of patients with alopecia areata and vitamin D deficiency were in the 21-30 age group (n=29; 50%), followed by the 31-40 age group (n=11; 19%). The distribution was similar in the control group. There was no significant difference in the age distribution of vitamin D deficiency/insufficiency between cases and controls (p-value = 0.138) (table 8, figure 8). No significant difference was seen between gender with respect to vitamin D deficiency (p value= .63).
- The mean Vitamin D levels were assessed in relation to the duration of alopecia areata. Patients with a history of the disease for three months

or less had mean Vitamin D levels of 21.49 ± 11.27 ng/ml. Those with a history of 3 to 6 months had a mean of 19.76 ± 9.8 ng/ml, patients with 6.1 to 12 months had a mean of 12.39 ± 6.4 ng/ml, and those with over 12 months had the lowest mean of 10.77 ± 0.31 ng/ml. We observed that as the duration of alopecia areata increased, the levels of Vitamin D tended to decrease; however, these results were statistically non-significant (p value= .132).

- The mean Vitamin D levels were highest in patients with alopecia areata affecting the eyebrows, with a mean of 23.27 ± 9.73 ng/ml. This was followed by hair loss at the beard, with a mean Vitamin D level of 22.82 ± 21 ng/ml, and scalp involvement, with a mean Vitamin D level of 19.6 ± 10.54 ng/ml. The lowest mean Vitamin D levels were observed in patients with whole-body involvement, at 11.90 ± 5 ng/ml.
- When assessing the diagnosis of Vitamin D levels and the pattern of alopecia areata, there was no statistical significance (p-value =.156). However, all patients with sufficient Vitamin D levels had patchy alopecia areata (AA). None of the patients with alopecia totalis, alopecia universalis, or diffuse alopecia areata had sufficient Vitamin D levels. Among the 58 patients with Vitamin D deficiency, 48 (82.8%) had patchy AA, 3 (5.2%) had alopecia totalis, 4 (6.9%) had alopecia universalis, and 3 (5.3%) had diffuse AA (Table 12a, figure 12a).
- However, the comparison of mean Vitamin D levels with the pattern of alopecia areata was statistically significant (p-value = .038). Patients with patchy alopecia areata had the highest mean Vitamin. D levels at 21.38 ± 11.02 ng/ml, followed by those with diffuse alopecia areata at 19.78 ± 8.30 ng/ml. Patients with alopecia universalis and alopecia totalis had lower mean Vitamin D levels at 9.67 ± 1.48 ng/ml and 8.27 ± 2.57 ng/ml, respectively. These results indicate a statistically significant difference in Vitamin D levels based on the pattern of alopecia areata.
- Among the 22 patients who had nail changes, 16 (27.6%) had a deficiency of Vitamin D, while 5 (23.8%) had insufficient Vitamin D levels

(Table 13a, figurea). The mean Vitamin D level in patients with nail changes was 16.01 ± 8.91 ng/ml, significantly lower than patients who did not have nail changes, with a mean of 21.66 ±11.15 ng/ml.

- The SALT score was limited in 44 cases, with a mean Vitamin D level of 21.83 ± 11.26 ng/ml. In moderate cases (n=16), the mean Vitamin D level was 15.29 ± 7.45 ng/ml, while in severe cases (n=5), it was 19.34 ± 6.18 ng/ml. Very severe cases (n=7) had a mean Vitamin D level of 9.07 ± 1.97 ng/ml (Table 14, figure 14). This association was found to be statistically significant (p value-.007). A mild inverse correlation was observed between SALT scoring and Vitamin D levels, implying that as the SALT score increases, Vitamin D levels mildly decrease. This correlation was statistically significant (r = -0.366; p = 0.002).

CONCLUSION

In conclusion, while our study provides compelling evidence of a link between vitamin D levels and AA, further research is essential to confirm these findings and to explore the potential therapeutic benefits of vitamin D. This could ultimately lead to improved management strategies for AA, benefiting patients with this challenging and often distressing condition. While our study provides valuable insights into the potential link between vitamin D levels and alopecia areata (AA), several limitations must be acknowledged.The relatively small sample size may limit the generalizability of our findings, and the cross-sectional design only provides a snapshot in time, preventing causal inferences. The study relied on a single measurement of serum 25(OH)D levels, which can fluctuate. The diversity of the study population was limited, potentially affecting the applicability of the results to broader groups. The subjective nature of the SALT score for assessing AA severity introduces potential bias. Other potential biases, including selection and information bias, may also have influenced the results. These limitations highlight the need for further research with larger, more diverse cohorts, longitudinal designs, and better control for confounding factors to validate and extend our findings.

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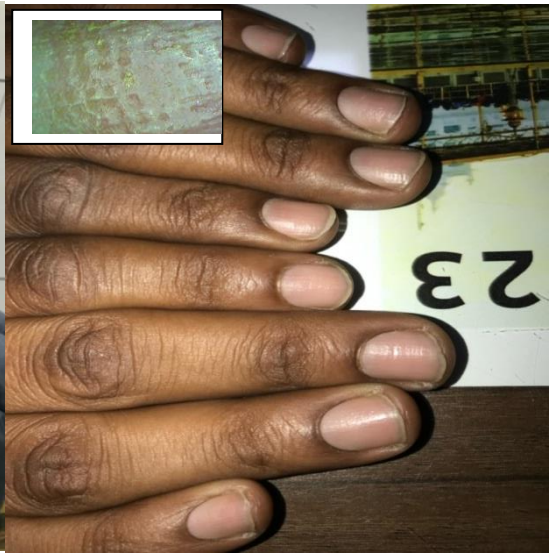
Pictue 1 : large patch of Alopecia Areata over Scalp

Pictue 2 : Single well circumscribed Patch of AA with smooth surface present over scalp

(a)

(b)

(c)



(d)

Pictue 3 :

- (A,B,C).Alopecia universalis
- (D.)Geometrical nail pitting was present

(a)

(b)

(c)

Picture 4:

- (A,B)Alopecia totalis
- (C)Beau's lines were present

(a) (b)

Picture 5: Alopecia Areata : (a) single large patch
(b) two large patches

Picture 6: Single well circumscribed patch of AA over Scalp in patient of androgenetic alopecia

Picture 7: Alopecia Areata : three patches of AA over Beard

Picture 8: Alopecia Areata : Left Eyebrow

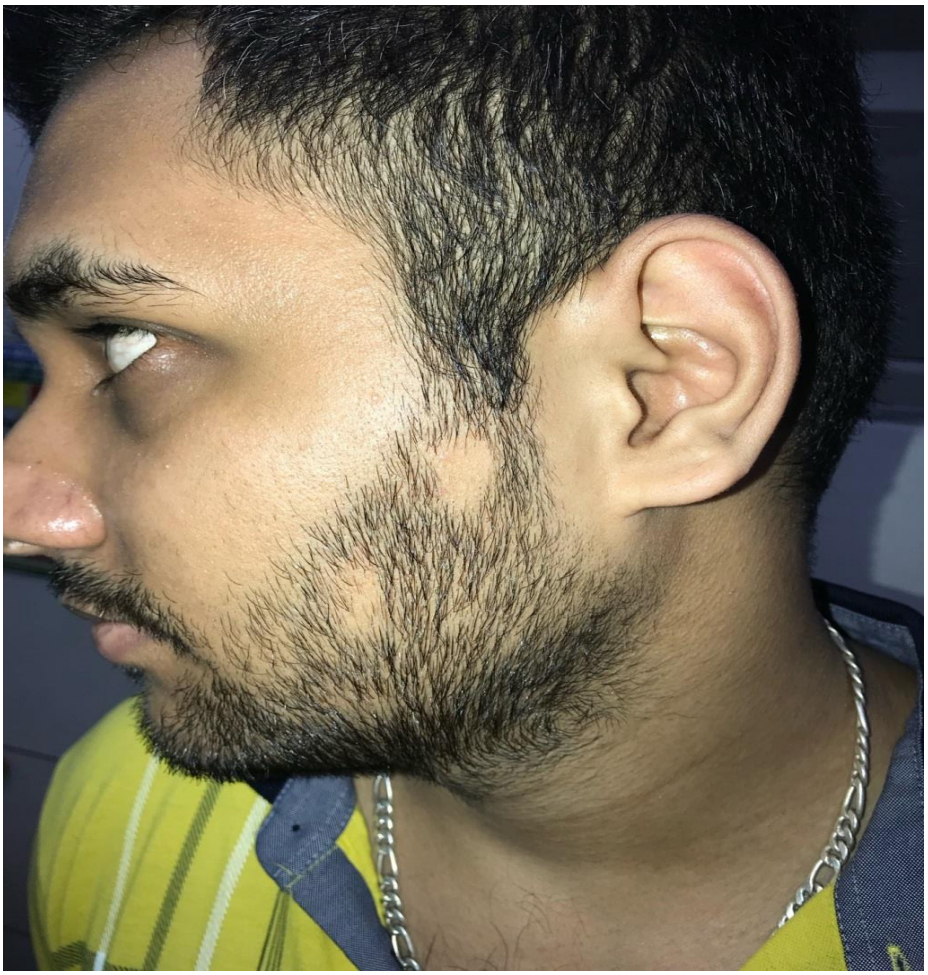
(a) (b)

(c)

Picture 9:

- (A, B) Alopecia Totalis
- (B)Beau's lines were present

Picture 10: Single large patch of AA over Beard



Picture 11: Alopecia areata (Beard) Number of patches = 2

Picture 12: Patchy AA in 25 year old atopic male

Picture 13: Alopecia Areata in a 42 years old female

KEY TO MASTER CHART

sex	1	Male
	2	Female
1st episode or relapse	1	First episode
	2	relapse
Site of hair loss	1	Scalp
	2	Beard
	3	Eyebrows
	5	full body
	7	scalp+ beard
	8	eyebrows +beard
	9	scalp + eyebrows
Pattern of hair loss	1	Patchy
	2	Alopecia totalis
	3	Alopecia universalis
	4	Diffuse alopecia areata
vitamin D	1	deficiency,
	2	insufficiency
	3	sufficiency

S. NO.	Groups	MRD NO	Age	sex(1=male, 2=female)	duration of illness (months)	1st episode or relapse (1=first episode, 2=relapse)	family history	H/o atopy	site of hair loss	pattern of hair loss	exclamation mark	Nail changes	pattern of nail changes	SALT score for severity	SALT % hair loss (AA-IGA BANDS	Serum,25 (OH) Vita,in D levels	vitamin D groups(1=deficiency,2=insufficiency,3=sufficiency)
1	1	HK 1456717	28	2	3	1	no	no	1	1	no	no		30	limited	18.31	1
2	1	SK 1548723	30	2	2	1	no	no	1	1	no	no		40	limited	22.23	2
3	1	KS 1470811	30	1	3	1	no	no	5	3	no	yes	geometrical pitting	100	very severe	11.29	1
4	1	P 1445769	29	2	2	1	no	no	1	1	yes	no		4	limited	16.01	1
5	1	NK 1467899	38	1	3	1	no	no	1	1	no	no		16	limited	15.3	1
6	1	NR 271530	34	1	1	1	no	no	1	1	no	no		25	moderate	6.15	1
7	1	AS 211423	58	1	4	1	no	no	1	1	no	no		12	limited	30.6	3
8	1	RS 1419312	27	1	6	1	no	no	5	3	no	yes	geometrical pitting	100	very severe	8.4	1
9	1	S 1466643	25	1	2	2	no	no	1	1	no	no		19.2	limited	3.79	1
10	1	SK 1543895	45	2	2	1	no	no	1	1	yes	no		43.6	moderate	18.93	1
11	1	NK 1738947	68	1	2	1	no	no	8	1	no	yes	geometrical pitting			27.7	2
12	1	MS 1519464	19	2	3	1	no	no	1	1	no	no		9	limited	29.7	2
13	1	NR 1538268	40	2	3	2	no	no	9	1	no	no		9	limited	24.1	2
14	1	SM 855794	40	1	3	1	no	no	1	1	yes	no		21.8	moderate	16	1
15	1	JS 1375174	24	1	3	2	no	no	2	1	no	no				12.58	1
16	1	SS 1547581	30	1	3	1	no	yes	2	1	no	no				17.27	1
17	1	VS 1547578	30	1	3	1	no	no	1	1	no	no		19.2	limited	28.32	2
18	1	GS 1530464	35	1	6	2	no	no	1	1	yes	yes	beau's lines	13.8	limited	23.31	2
19	1	HS 1548365	29	1	1	1	no	no	2	1	no	no				35.43	3
20	1	SS 1536229	26	1	2	1	yes	no	2	1	no	no				9.14	1
21	1	SB 1550896	24	2	1	1	no	no	9	1	no	no		10.4	limited	17.76	1
22	1	MK 1522172	36	2	6	1	yes	no	1	1	no	no		9.6	limited	27.96	2
23	1	HK 1456716	28	2	3	1	no	no	1	1	no	no		10.6	limited	17.96	1
24	1	SK 1548723	30	2	2	2	no	no	1	4	no	yes	beau's lines	18.6	limited	27.91	2
25	1	SK 7918	40	1	3	1	no	yes	1	1	no	no		16.2	limited	5.1	1
26	1	RS 826943	27	1	1	2	no	no	2	1	no	no				33.36	3
27	1	PK 1468827	24	1	1	1	no	no	1	1	no	no		5.4	limited	13.92	1
28	1	SS 1543255	37	1	3	2	no	no	2	1	no	no				27.96	2
29	1	A 1542751	26	1	1	2	no	no	1	1	no	no		13.8	limited	37.91	3
30	1	KK 1468650	28	2	3	1	no	no	3	1	no	no				37.9	3
31	1	H 1584871	32	2	1	1	no	no	3	1	no	no				37.41	3
32	1	P 1563007	29	2	2	2	no	no	1	1	no	no		3.6	limited	31.6	3
33	1	S 1593472	27	2	6	2	no	yes	1	1	no	no		9.6	limited	31.4	3
34	1	NT 1567686	57	2	2	1	no	no	1	1	no	no		11.6	limited	38.22	3
35	1	VG 175736	76	1	3	2	no	no	1	1	no	yes	beau's lines	10.4	limited	39.41	3
36	1	MB 1376243	25	2	6	1	yes	yes	1	1	yes	no		7.2	limited	7.58	1
37	1	RM 4640	51	2	1	1	yes	yes	1	1	no	no		15.2	limited	17.91	1
38	1	M 812508	30	1	3	1	yes	no	1	4	no	no		38.4	moderate	24.71	2
39	1	PS 327869	33	1	3	1	no	no	1	1	yes	no		13.6	limited	21.44	2
40	1	SK 1487078	31	1	6	2	no	no	1	4	no	yes	geometrical pitting,beau's lines	51.2	severe	10.83	1
41	1	G 895526	19	2	1	1	no	yes	3	1	no	no				10.62	1
42	1	AS 1572175	47	1	4	1	no	no	1	1	yes	no		28	moderate	34.71	3
43	1	RB 787190	37	1	9	1	no	no	1	4	no	no		58	severe	17.71	1
44	1	H 1498828	26	1	2	1	no	no	1	1	no	no		16	limited	3.89	1
45	1	D 1130151	20	2	6	1	yes	no	1	2	no	yes	geometrical pitting, punctuate leukonychia	100	very severe	7.91	1
46	1	SS 338405	46	2	1	1	no	no	1	1	no	no		8	limited	11.02	1
47	1	KK 1538229	21	2	2	1	no	no	3	1	no	no				14.31	1
48	1	ST 1578298	34	1	5	1	no	no	2	1	no	no				34.91	3
49	1	P 1558480	64	2	4	1	no	no	1	1	no	no		16	limited	17.07	1
50	1	AK 1545738	29	1	4	1	no	no	2	1	no	no				9.95	1
51	1	AS 1229882	19	1	3	1	no	no	1	1	yes	no		18	limited	24.82	2

S. NO.	Groups	MRD NO	Age	sex(1=male, 2=female)	duration of illness (months)	1st episode or relapse (1=first episode, 2=relapse)	family history	H/o atopy	site of hair loss	pattern of hair loss	exclamation mark	Nail changes	pattern of nail changes	SALT score for severity	SALT % hair loss (AA-IGA BANDS	Serum,25 (OH) Vita,in D levels	vitamin D groups(1=deficiency,2=insufficiency,3=sufficiency)
52	1	RS 1540405	24	1	1	1	no	no	1	1	no	no		23	moderate	11.89	1
53	1	HS 1484263	30	1	2	1	no	no	2	1	no	no				17.91	1
54	1	RS 224971	26	1	2	2	no	no	7	1	yes	yes	geometrical pitting	47	moderate	14.89	1
55	1	MS 1450844	40	1	2	2	no	yes	1	4	no	yes	geometrical pitting	42	moderate	9.61	1
56	1	RA 228326	42	1	1	1	no	no	1	4	no	yes	beau's lines	59	severe	27.9	2
57	1	MK 1462840	30	2	2	1	no	yes	1	1	no	no		13	limited	7.05	1
58	1	MK 1468699	62	1	2	2	no	no	1	1	no	no		24	moderate	13.83	1
59	1	JK 8117	35	2	2	1	yes	no	1	1	no	no		12	limited	44.06	3
60	1	RS 854076	25	1	6	1	no	yes	1	1	no	no		28	moderate	15.83	1
61	1	AA 1489012	39	1	1	1	no	no	1	1	no	no		7	limited	32.11	3
62	1	SY 384584	28	1	3	1	no	no	8	1	no	no				34.31	3
63	1	PS 1490078	21	1	1	1	no	yes	1	1	yes	no		18.9	limited	20.57	2
64	1	AD 828302	44	2	2	2	no	no	1	1	no	yes	beau's lines	15.31	limited	13.41	1
65	1	ST 325109	19	1	4	1	yes	no	1	1	no	no		13	limited	24.81	2
66	1	G 1416053	22	1	12	1	no	no	1	2	no	yes	geometrical pitting	100	very severe	5.89	1
67	1	A 1428762	32	1	5	1	no	no	1	1	no	no		8	limited	27.62	2
68	1	RK 652186	44	1	1	1	no	no	1	1	yes	no		13	limited	16.39	1
69	1	R 1575523	19	2	2	1	no	no	3	1	no	no				5.76	1
70	1	HK 1576975	27	2	1	1	no	no	1	1	no	no		47	moderate	7.98	1
71	1	KS 1403949	30	1	2	1	no	no	5	1	no	yes	geometrical pitting	59	severe	21.35	2
72	1	JM 1677623	32	1	3	1	no	no	7	1	yes	yes	beau's lines	32	moderate	13.83	1
73	1	H 1605139	30	2	2	2	no	yes	3	1	no	no				17.77	1
74	1	HS 4435	21	1	1	1	no	no	1	1	no	no		13	limited	14.02	1
75	1	AG 1692418	19	1	2	1	no	no	1	1	no	no		12	limited	8.02	1
76	1	RS 1270997	44	2	3	1	no	no	3	1	no	no				22.47	2
77	1	AS 1657000	56	1	12	2	no	no	8	1	no	yes	geometrical pitting			5.4	1
78	1	RK 676566	30	2	3	1	no	no	1	1	yes	no		46	moderate	7.8	1
79	1	CV 1566493	32	1	2	1	no	no	1	1	no	no		6	limited	10.82	1
80	1	SS 1547587	30	1	3	1	no	no	2	1	no	no				17.27	1
81	1	HS 1565536	27	1	2	1	no	no	2	1	no	no				19.18	1
82	1	SK 1543095	45	2	8	1	no	no	1	1	no	yes	geometrical pitting , trachonychia	55	severe	18.93	1
83	1	SK 1696850	21	1	2	1	no	no	1	1	no	no		7	limited	21.2	2
84	1	SS 1683857	34	1	1	2	no	no	2	1	no	no				39.44	3
85	1	SS 1552938	25	1	3	1	no	yes	1	1	no	no		9	limited	44.61	3
86	1	DG 379346	55	2	1	1	no	no	3	1	no	no				36.17	3
87	1	HS 1009711	39	1	3	2	no	no	2	1	no	no				27.4	2
88	1	VA 1714870	35	1	2	1	no	no	2	1	no	no				19.11	1
89	1	GS 1712114	43	1	6	1	no	no	1	1	no	no		14	limited	15.34	1
90	1	DP 1646580	20	2	4	1	no	no	5	1	no	yes	geometrical pitting	25	moderate	7.93	1
91	1	RD 1635989	42	2	7	2	no	no	3	3	no	yes	geometrical pitting,punctuate leukonychia	100	very severe	8.42	1
92	1	MK 1659943	32	1	1	1	no	no	1	1	no	no		13	limited	47.78	3
93	1	AR 1651253	35	1	60	1	yes	no	5	3	no	yes	geometrical pitting	100	very severe	10.55	1
94	1	PS 1658440	49	1	36	1	no	no	1	2	no	no		100	very severe	11	1
95	1	AV 1494274	27	1	3	1	no	no	2	1	no	no				36.11	3
96	1	PK 1570691	31	1	2	1	no	no	2	1	no	no				26	2
97	1	S 1572780	23	1	3	1	no	no	3	1	no	no				44.31	3
98	1	SB 1550096	44	2	4	1	no	no	9	1	no	yes	beau's lines	17	limited	19.56	1
99	1	SV 1539539	25	1	2	1	no	no	1	1	no	no		4	limited	11.15	1
100	1	K 583426	28	2	8	2	no	no	1	1	no	yes	punctuate leukonychia	12	limited	18	1

S. NO.	Groups	MRD NO	Age	sex(1=male, 2=female)	Serum,25 (OH) Vita,in D levels	vitamin D groups(1=deficiency,2=insufficiency,3=sufficiency)
1	2	GC 1527181	50	1	31.33	3
2	2	AS 1150417	33	1	34.31	3
3	2	SB 251139	25	2	20.72	2
4	2	PS 597068	35	1	13.1	1
5	2	JK 1138425	61	2	16.1	1
6	2	UK 350103	70	2	60.78	3
7	2	JK 440556	23	2	26.4	2
8	2	RS 1508893	30	1	21.41	2
9	2	A 265515	49	2	34.83	3
10	2	JK 1507977	19	2	8	1
11	2	BK 1401624	23	1	4.5	1
12	2	CK 1472651	22	2	7.5	1
13	2	NJ 1498261	36	2	31.98	3
14	2	SK 1450060	60	2	25.38	2
15	2	K 1470897	25	2	6.96	1
16	2	SJ 1244784	31	1	15.98	1
17	2	SK 5925	55	2	38.69	3
18	2	KP 1364428	25	1	39.2	3
19	2	SK 1491147	24	2	10.29	1
20	2	VK 14870791	33	1	8.7	1
21	2	DS 1543154	25	1	34.2	3
22	2	P 231610	22	2	14.38	1
23	2	RC 1092986	58	2	31.3	3
24	2	ID 1452123	67	2	47.88	3
25	2	AA 1531340	22	2	27.6	2
26	2	KK 1538229	21	2	36.88	3
27	2	GS 159093	36	1	45.7	3
28	2	L 1560597	26	2	35.36	3
29	2	NR 1538268	40	2	24.1	2
30	2	SS 1416437	19	2	70.21	3
31	2	RS 1270997	44	2	42.1	3
32	2	LK 1651383	39	2	38.33	3
33	2	S 22572	37	2	46	3
34	2	SD 1661148	45	2	32.19	3
35	2	RK 121005	50	2	18.03	1
36	2	PS 1544159	20	1	54.91	3
37	2	AK 1430820	48	1	15.6	1
38	2	JK 1667030	19	2	28.11	2
39	2	NR 1691907	32	2	35.37	3
40	2	R 1713527	46	2	28.21	2
41	2	PD 1643778	57	2	21	2
42	2	AS 1647996	55	1	39.11	3
43	2	SS 1567426	45	1	9.54	1
44	2	R 207524	36	2	78.7	3
45	2	GK 1548986	42	2	23.2	2
46	2	TN 1536013	19	2	35.28	3
47	2	MS 1367436	36	1	27.3	2
48	2	GK 1579021	33	2	51.55	3
49	2	R 1028556	34	2	36.56	3
50	2	PK 23491	39	2	19.4	1

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The Principal
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Subject:: Approval of Plan of Thesis.

This is to inform you that the Faculty of Medical Sciences in its meeting held on 25.09.2023 has approved the plan of thesis of **Stephen**, a student of your college for **MD Dermatology** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
A study of vitamin D levels in patients of alopecia areata .

Supervisor
Dr. Sukhjot Kaur
Assoc. Prof.,
Dept. of Dermatology

Co-Supervisor:-
Dr. Sunil K Gupta
Prof. & Head,
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Co-Supervisor:-
Dr. Ekta Bansal
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The student may submit the thesis in triplicate alongwith the plan in triplicate also, to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She may be directed to attach a photocopy of this letter with the forwarding letter while submitting thesis in the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic, if any.

Controller of Examinations

Note :

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN

PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MD

In

DERMATOLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

August, 2022

DR. STEPHEN

DEPARTMENT OF DERMATOLOGY

DAYANAND MEDICAL COLLEGE AND HOSPITAL

LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA
For the degree of	MD (DERMATOLOGY)
Name of the candidate	Dr. STEPHEN
Supervisor	Dr. Sukhjot Kaur, Associate Professor, Department of Dermatology, Venerology and Leprosy, DMCH , Ludhiana
Co-supervisor(s)	Dr. Sunil K. Gupta, Professor & Head, Department of Dermatology, Venerology and Leprosy, DMCH , Ludhiana Dr. Ekta Bansal, Professor & Head, Department of Biochemistry, DMCH, Ludhiana
Institution	Dayanand Medical College and Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Alopecia areata (AA) is the most common form of non scarring alopecia involving any hair bearing area. AA is an organ-specific autoimmune disease characterized by T-cell infiltrates and cytokine production around anagen stage hair follicle. Vitamin D has been hypothesized as a risk factor for the development of AA. Vitamin D plays an important role in the pathogenesis of AA.

Aim of Study: To Study the Co-relation of Vitamin D levels in patients of Alopecia areata

Materials & methods: 100 Patients of alopecia areata age 18 years or above attending skin and VD OPD of Dayanand Medical College & Hospital, Ludhiana will constitute study material. 50 age and Gender-Matched controls will include healthy individuals known to have no effect on vitamin D levels and their 25(OH)vitamin D levels will be estimated. grading will be done based on SALT's Score.

Clinical Significance: To find out whether low Sr. Vitamin D levels are more frequent in patients with AA in comparison to healthy controls.

Keywords: Alopecia areata
25(OH)Vitamin D

(Signature)	(Signature)	(Signature)	(Signature)	(Signature)
Candidate	Co-supervisor	Co-supervisor	Supervisor	Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA"** exist in the Department of Dermatology, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate himself under our supervision and guidance.

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Date : 17/9/22

APPROVAL PROFORMA
BY RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Stephen
Department	Dermatology
Topic of Thesis	A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA
Likely date of appearing in PG examination	May/June 2024
Date of enrollment	7th March, 2022
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Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Harpreet Kaur (Professor & Head, Pathology)	Mr. R K Sharma (Legal Expert)
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Approved: Yes / ~~No~~

Dr. Sandeep Puri
Chairperson
Research Committee

Chairperson
Research Committee

Approved: Yes / ~~No~~

Prof. Arvind Malhotra
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BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	Dr. Stephen 9/410/4 Partap nagar, Banga Distt. SBS Nagar
2	NAME OF THE INSTITUTE	Dayanand Medical College and Hospital, Ludhiana
3	COURSE OF STUDY AND SUBJECT	M.D. Dermatology, Venerology & Leprology
4	DATE OF ADMISSION OF COURSE	7th March, 2022
5	TITLE OF THE TOPIC	A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Alopecia areata (AA) is the most common form of nonscarring alopecia that involves any hair bearing area. It was first described by Cornelius Census, and the term “alopecia areata” was coined by Savages in 1760.[1] The prevalence of AA is 0.1%–0.2% with a calculated lifetime risk of 2% affecting children and adults also hair of all colors.[2] The disease is shown to be severe in males and those who are affected in early childhood. Paediatric AA constitutes about 20% of AA cases. 60% of AA patients had their first patch before the age of 20 years.[3] AA is an organ-specific autoimmune disease characterized by T-cell infiltrates and cytokine production around anagen stage hair follicle.

AA is associated with human leukocyte antigen – Class I and II are known to occur with various autoimmune disorders such as rheumatoid arthritis, diabetes mellitus, vitiligo, systemic lupus erythematous, thyroiditis, pernicious anaemia, and coeliac disease.

Hair follicle-specific autoantibodies are increased in peripheral blood of AA patients, especially to keratin 16 and trichohyalin.

Thus, AA is considered as hair follicle-specific autoimmune disease and is triggered by environmental factors in genetically susceptible individuals.

Nail changes are seen in about 29% of adults and 50% of children with AA. They are more common in males and in severe AA. Nail changes may precede or follow the hair loss limiting to one or more nails. Nail changes typical of AA are geometric pitting (multiple, small, and superficial pits regularly distributed along transverse and longitudinal lines), geometric punctate leukonychia (multiple white spots in a grill pattern), and trachyonychia (sandpaper nails).

Vitamin D is a secosteroid hormone that plays an important role in calcium homeostasis and bone health. Vitamin D has been hypothesized as a risk factor for the development of AA. Vitamin D plays an important role in the pathogenesis of AA. Evidence has arisen from the reports of AA in patients with hereditary Vitamin D-resistant rickets. Further evidence is provided by the effect of Vitamin D on

protecting hair follicles from chemotherapy-induced alopecia. Expression of Vitamin D receptors in keratinocytes is necessary for the maintenance of normal hair cycle. 1, 25-dihydroxyvitamin D3 acts as an immune modulator targeting various immune cells such as monocytes, macrophages, dendritic cells, as well as T lymphocytes and B lymphocytes, thus modulating both innate and adaptive immune responses.

It has been suggested that Vitamin D and its analogs not only prevent the development of autoimmune disease but could also be used to treat them. In view of emerging evidence of association between AA and Vitamin D deficient levels, it is imperative to revaluate the correlation between AA and Vitamin D levels for better management of this chronic disease.

6.2 REVIEW OF LITERATURE

Alopecia areata (AA) is a common autoimmune skin disease characterized by loss of the hair on the scalp and elsewhere on the body, affecting approximately 2% of the general population at some point during their lifetime. AA may cause anxiety on patients and increases the risks of developing psychological and psychiatric complications.[4] The hair loss in AA is believed to result from an autoimmune-mediated hair follicle (HF) destruction consequent to a loss of immune privilege (IP) in the HF. Autoreactive effector T cells and mast cells, CD8-positive nature killer group 2 member D (NKG2D)-positive cytotoxic T cells ($CD8^+NKG2D^+$ cytotoxic T cells), Janus kinase/signal transducers and activators of transcriptional signaling (JAK/STAT) pathways, regulatory T cells (Tregs) and immune checkpoints and oxidative stress (OS) are involved in AA.[4,5] However, the pathogenesis of AA remains incompletely understood and AA remains incurable.

Vitamin D has been associated with various autoimmune diseases. Recently, vitamin D deficiency has been reported in AA.[6] Moreover, topical calcipotriol has been reported to be used successfully in treating AA.[7-9] These reports attracted the attention on the relationship between vitamin D and AA. The aim of this review is to summarize the current knowledge on the possible roles of vitamin D in the pathogenesis of AA and to discuss the potential implication of vitamin D in the treatment of AA.

Potassium hydroxide smear, fungal culture, serology for syphilis, and scalp biopsy may help in doubtful cases. Hair pull test, hair pluck test, dermoscopy, SALT score (severity of alopecia tool score) are useful in assessing the activity and severity of the disease. Optical coherence tomography (OCT) is a recently evaluated non-invasive technique to detect the hair shaft abnormalities in AA. Bartles *et al*. demonstrated that the cross section of hairs from an AA patch was significantly lower compared with hairs of an unaffected area by this OCT. Thus, OCT may be an useful non-invasive technique to differentiate AA from other

causes of patchy alopecia, such as trichotillomania. Presence of exclamatory mark hairs at periphery, positive hair pull test (>6 hairs), daily hair count (>100 hairs), hair pluck test (more telogen hairs) and dermoscopy (black dots, brokenhair, and tapering hair) suggest active disease. Severity of AA can be measured by SALT score, developed by the National Alopecia Areata Foundation working committee.

SALT Score

SALT score is useful to find out the quantitative assessment of scalp hair loss. The entire scalp is divided into 4 parts based on the surface area, top (40% - 0.4), posterior (24% - 0.24), right side (18% - 0.18), and left side of scalp (18% - 0.18). Percentage of hair loss in each area is determined independently and is multiplied by the percentage of scalp covered in that area of the scalp, and summing the products of each area will give the SALT score. For example, the hair loss is 40%, 30%, 20% and 10% in top, back and right and left side respectively, then the SALT score can be calculated as- (40 × 0.4) + (30 × 0.24) + (20 × 0.18) + (10 × 0.18) =16 + 7.2 + 3.6 + 1.8 = 28.6. SALT score is easily reproducible and validated. However, it does not include hair pigmentation, body hair, and nail involvement.

Vitamin D sources, metabolism and functions

In the human body there are two major forms of vitamin D. More than 90% is vitamin D_3 (cholecalciferol), which is converted from 7-dehydrocholesterol by ultraviolet light B (UVB) exposure in the skin. About 10% is vitamin D2 (ergocalciferol), which comes from dietary sources. Both are biologically inactive.[10] In the liver, vitamin D is metabolically converted into 25-hydroxyvitamin D (25(OH)D) which is biologically inactive at physiological concentration. 25(OH)D has been considered as one of the most reliable indicator of Vitamin D levels in humans.[11] In the kidney, 25(OH)D is further converted to the biologically active metabolites $1,25(OH)_2D_3$ (calcitriol) by 25(OH) 1α-hydroxy-D.[12] The 1α-hydroxy enzyme is also widely expressed in non-kidney cells including immune cells and is able to convert the inactive

25(OH)D into the active 1,25$(OH)_2$D in either an autocrine or paracrine manner.[12]

Vitamin D functions by binding to the vitamin D receptor (VDR), a member of nuclear hormone receptors which is widely expressed in the kidney, immune cells, osteocytes and other types of cells. VDR activated by vitamin D forms a heterodimeric complex with retinoid X receptor. This complex is recruited to the vitamin D response elements in the target genes and interacts with additional co-regulators, influencing the expression of many genes. As such, vitamin D possesses multiple functions and target organs.[10,13]

At present, the function of vitamin D can be divided into two categories. The classical function type which involves mineral balance and skeletal maintenance. The non-classical function type which involves regulation of cellular proliferation, differentiation, apoptosis and adaptive immunity, anti-oxidation and others. The role of vitamin D in many other medical conditions such as cancer, diabetes, hypertension, cardiovascular, and autoimmune and dermatological diseases is being extensively studied.[10,13]

Vitamin D status in AA

A number of studies demonstrated significantly lower levels of vitamin D in the patients with AA than the control group.[14-20] Several studies showed significantly higher prevalence of vitamin D insufficiency in patients with AA than the control group.[15,16,21,22] But there were two reports of inconsistent results. A Turkish study found that AA patients had a deficiency of 25(OH)D, but there was no statistically significant difference in the serum vitamin D levels between AA patients and healthy controls. The authors said that this might be due to the universal tendency toward lower values of 25(OH)D in their geographical area, and they noted that the blood samples were collected only once during the late fall and winter months.[23] In a study involving 55,929 women in the Nurses' Health Study, 133 cases of AA were identified over a follow-up of 12 years. The association between estimated vitamin D status and self-reported incident AA was prospectively evaluated. No significant association between a

predictive score of serum 25(OH)D levels and risk of incident AA was found.[24] Importantly, two systemic reviews and meta-analyses published in 2018 did demonstrate that, patients with AA have a higher prevalence of vitamin D deficiency and lower vitamin D levels than the control group.[6,25] Moreover, several studies revealed that serum vitamin D levels significantly and inversely correlate with the severity of AA.[15,19,20,22,26]

Vitamin D status in AA.

Study was done by Yilmaz et al in 2012 in turkey using case control method in 42 patients which concluded that there was lower serum vitamin D levels were seen.

In 2014, Mahamid et al studied in 23 patients in Isreal and found lower serum vitamin D levels in patients with alopecia areata.

Lee et al in 2018 did systematic review &meta-analysis and concluded that AA patients have higher vitamin D deficiency prevalence and lower serum vitamin D than controls.

Bhat et al, in India did a cross-sectional study in 2017 and found lower serum vitamin D levels.

Studies also showed that serum [27] and tissue [22,27] VDR levels were lower in AA. One study found a negative correlation of tissue VDR and extent of AA.[27]

Taken together, the above data show a substantial link between the levels of vitamin D and AA, suggesting an important role of vitamin D in the pathogenesis of the disease. However, the mechanism underlying this relationship still has to be deciphered

Possible role of vitamin D in pathogenesis of AA

Hair loss in AA is caused by the destruction of HF cycle. However, the role of vitamin D in in HF cycling is not clear. Although a study showed in mice that in calbindin-D_{9k} knockout pups, a maternal vitamin D-deficient/low-calcium diet leads to transient noncicatricial alopecia, suggesting a role for calcium and possibly vitamin D in postnatal HF cycling, studies published in the literature have not demonstrated a role of vitamin D in HF cycling. On the other hand, it is now fully accepted that VDR has important function in hair cycling. However, this function

of VDR appears to be vitamin D-independent . VDR may function as a selective suppressor/de-repressor of gene expression in the absence of 1,25$(OH)_2D_3$. Wnt/β-catenin signaling is a key player in inducing the onset of anagen and maintaining the cycling transition during the initiation and regeneration of HFs .Reduction of VDR expression in AA may be related to decreased hair cycle-related signals-Wnt/β-catenin signals .The decreased expression of VDR in AA is believed to be involved in the disruption of HF cycling in AA.

The disruption of HF cycling in AA is caused by an autoimmune response, and there is mounting evidence that vitamin D has an impact on the pathophysiological mechanisms of autoimmunity . Therefore vitamin D may affect the HF cycling through its impact on autoimmunity in AA pathogenesis.

Possible roles of Vitamin D in the pathogenesis of alopecia areata focussing on several key elements of autoimmune mechanisms have been reviewed.

Loss of IP in HF

The anagen HF is normally an immune privileged (IP) site. The loss of the IP of the anagen hair bulb is a key element in AA pathogenesis. One of the most crucial mechanisms of IP collapse is an increase in major histocompatibility complex (MHC) I and II molecules .IFN-γ is prominently expressed in lesional skin from patients with AA and is believed to contribute to the collapse of IP through increased follicular expression of MHC class I and II molecules.

Studies revealed that vitamin D_3 significantly inhibits the production of IFN-γ by human peripheral blood mononuclear cells (PBMCs) activated in vitro by phytohemagglutinin and decreases the secretion of IFN-γ by human $CD4^+$ T cells .Kokic et al showed that in patients with inactive SLE, lower levels of vitamin D are corelated with higher levels of IFN-γ. In vitamin D deficient patients, IFN-γ was 150% higher compared with patients without vitamin D deficiency. Mrad et al investigated the immunologic effects of vitamin D replacement in relapsing remitting multiple sclerosis (RRMS) patients. The patients categorized as vitamin D deficient were treated with high dose vitamin D (10,000 IU orally

daily for 3 months), while those with normal vitamin D maintained their usual medical care. They observed a decreased IFN-γ secretion by $CD4^+$ T cells in vitamin D deficient group but not in the sufficient group, and a negative correlation between baseline serum vitamin D and IFN-γ production. These findings suggest that normally vitamin D might contribute to maintain the IP of HF by modulating the production of IFN-γ. Contrarily, insufficiency of vitamin D might lead to over-secretion of IFN-γ and play a role in the collapse of IP of the anagen hair bulb.

JAK/STAT pathway

Cytokines involved in the pathogenesis of AA, most notably interferon (IFN)-γ and interleukin (IL)-15, are dependent on the Janus kinase and signal transducers and activators of transcription (JAK-STAT) pathway. It is assumed that $CD8^+NKG2D^+$ T cells mediate AA in part through JAK signaling. Skin biopsy specimens from patients with AA displayed a strong JAK3 expression. JAK activation was also shown by the presence of phosphorylated STAT proteins in AA HF, but not in normal HF.

Studies have shown an inhibitory effect of vitamin D on the JAK/STAT pathway. Employing a mouse model of type 2 diabetes mellitus, Zhang et al observed that $25VD_3$-enhanced protein tyrosine phosphatase non-receptor type 2 expression decreased the expression of the JAK1/STAT3 signaling proteins in the gingival epithelium. Zeitelhofer et al showed in a functional genomics analysis of vitamin D effects on $CD4^+$ T cells *in vivo* in experimental autoimmune encephalomyelitis that JAK/STAT signaling pathway genes are down-regulated upon vitamin D supplementation. Inhibition of JAK/STAT pathway by vitamin D may in turn block the effects of key cytokines involved in the pathogenesis of AA.

These data suggest that vitamin D may contribute to control the overactive self-effector T cells in the pathogenesis of AA by enhancing the inhibitory function of Treg cells.

6.3 AIMS AND OBJECTIVE

TO STUDY THE CO-RELATION OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA

7. MATERIALS AND METHODS

100 Patients of alopecia areata of age 18 years or above attending skin and VD OPD of Dayanand Medical College & Hospital, Ludhiana will constitute study material .Informed Consent will be taken. Thorough clinical examination will be done. Alopecia areata will be graded based on SALT's Score.[28]

Blood samples will be taken and quantitative measurements of 25-hydroxyvitaminD levels will be done by Chemilumiscence immunoassay on Beckman Access 2 system. Vitamin D levels will be classified as: Deficient/Adequate according to values. 50 age and Gender-Matched controls will include healthy individuals, healthy attendants of patients, patients of the skin diseases known to have no effect on vitamin D levels and their vitamin D levels will be estimated .Findings will be tabulated, analysed statistically and differences in the two groups, if any will be noted. An attempt will be made to correlate levels of Vitamin D with extent of Alopecia areata.

7.1 SOURCE OF DATA:

INCLUSION CRITERIA :

1. Clinically diagnosed patients of age18 years or above with Alopecia areata
2. Patients willing to participate in the study.

EXCLUSION CRITERIA:

1. Concurrent or family history of autoimmune diseases – vitiligo, rheumatoid arthritis, diabetes, thyroid disorder, or lupus erythematosus
2. Vitamin D supplementation
3. Women wearing veil
4. Frequent ingestion of alcohol, gastrointestinal problems, bone or renal disease, or sarcoidosis or any other metabolic systemic diseases treated with systemic steroids, barbiturates, bisphosphonates, sulfasalazine, and psoralen with ultraviolet A/ultraviolet B therapy in the past 3 months
5. Pregnancy or lactation
6. Obesity defined by body mass index (BMI) ≥25
7. Patient denying to be a part of the study.

Number of Cases: 100

OUTCOME: Assessment of levels of vitamin D in patients of AA.

STATISTICAL ANALYSIS:

Data will be described in terms of range; mean and standard deviation (±SD%)frequencies (no. of cases) and relative frequencies (percentage) as appropriate. comparison of quantitative variables between the study groups will be done using student t test and Mann whiteney U test for independent samples for parametric and non parametric data respectively. Chi square test will be performed and exact test will be used when the expected value is less than 5.A probability value (P value) less than 0.5 will be considered statistically significant. All statistical calculations will be done using SPSS21 (Statistical package for social science) for microsoft windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

Yes, Vitamin D Levels.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes, Attached here with

8. LIST OF REFERENCES

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PROFORMA

Serial no: MRD no. : Date:

Name: Age: Sex:

Chief Complaints:

Duration of illness:

H/O any treatment taken previously:Yes/No

1st episode or relapse:

Family History:Yes/No

History of any other autoimmune disease:

History of Atopy:Yes/No

EXAMINATION:

Site of hair loss : Scalp/eyebrows/beard/body/genital

Pattern of Hair loss:-Patchy AA/Alopecia Totalis/Alopecia universalis/AA Ophiasis/AA Sisaipho/diffuse AA

Exclamation Mark : Y/N

Signature of Candidate **Signature of Supervisor**

Nail Changes : Yes/No

Pattern of nail changes: geometric pitting/ punctuate leukonychia/ trachonychia/ beau's lines/ onychomadesis/ other

SALT Score for severity:

Top (40%)	Back (24%)	Left Side (18%)	Right Side (18%)	Total % of Hair loss

Severity as per SALT Score:

SALT % Hair loss (AA-IGA BANDS):

-No hair loss= 0%

-Limited = 1-20%

-Moderate=21-49%

-Severe=50-94%

-Very severe=95-100%

Serum,25(OH) Vitamin D Levels :

Sr,25(OH)Vitamin D status	Value	Unit	Range
Deficient		ng/ml	<20
Insufficient		ng/ml	21-30
Sufficient		ng/ml	31-100
Toxicity		ng/ml	>100

Signature of Candidate **Signature of Supervisor**

INFORMED PATIENT CONSENT

Date: _____

I_____D/o,W/o,S/o_____.

am willing to participate in the study **"A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

…….. ……..

Signature of the Witness Signature of the patient

Name:

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____
निवासी _____
इस शोधकार्य **A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

“A STUDY OF VITAMIN D LEVELS IN PATIENTS OF ALOPECIA AREATA” ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ